New aspects of organophosphorus chemistry

Kawamoto, Aparecida Minhoko

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NEW ASPECTS OF ORGANOPHOSPHORUS CHEMISTRY

Submitted by

Aparecida Minhoko Kawamoto

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
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Dedicated to my mother, my sister Rosalina and my nephew Gustavo for their love and support.

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ABSTRACT

The two principal aims of this research work are the synthesis of new organodifluorophosphonates and new oxaziridine compounds.

The organodifluorophosphonates were hoped to have superior biological activity, compared to the natural systems. The oxaziridines were expected to behave as effective bonding agents in polymerization processes and possibly to be superior oxidizing reagents.

A new class of difluorovinylphosphonate analogues of PEP, methyl 2-[2',2'-difluoroethyl-2'-(diethoxyphosphinyl)] propenoate, methyl 2-[2',2'-difluoro ethyl-2'-(diethoxyphosphinyl)] propenoic acid, 2-(2',2'-difluoroethy-2'-(dihydroxy phosphinyl) propenoic acid, E-[4,4-difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid, and Z-[4,4-difluoro 4-(diethoxyphosphinyl)] but-2-enoic acid, have been synthesized.

The organodifluoro phosphonate, methyl [3,3-difluoro-3-(diethoxyphosphinyl) -2-hydroxy-2-methyl] propionate, and the corresponding acid derivative [3,3-difluoro-3-(dihydroxyphosphinyl)-2-hydroxy-2-methyl] propionic acid, have also been synthesized. These compounds were designed to act as potential inhibitors in the shikimic acid pathway.

[4,4-Difluoro-4-(dihydoxyphosphinyl)-2-amino] butanoic acid, an analogue of phosphoserine, has been synthesized by two different routes; one route gave a racemic mixture, and the other route, by using the strategy of coupling reaction with a chiral compound gave a single enantiomer.

The synthesis of oxaziridines was studied utilising hydroxylamine-O-sulfonic acid to produce oxaziridine unsubstituted on the nitrogen, which was reacted *in situ* with benzoyl chloride, isophthaloyl dichloride, and 1,3,5-benzenetricarbonyl trichloride, to give the corresponding 2-benzoyl-3,3-dimethyl oxaziridine, 1,3-benzene dicarbonyl-bis-(3,3-dimethyloxaziridine) and 1,3,5-benzenetricarbonyl-tris-(3,3-dimethyloxaziridine). These oxaziridines were tested in oxidative reactions of sulfides to sulfoxides, styrene to styrene oxide, cyclohexene to cyclohexene oxide, and 1-(2-cyclohexenyl)-2-propanone to [1-(2-cyclohexenyl)-2-propanone] oxide

ABBREVIATIONS

ADP	- adenosine diphosphate
AIBN	- azobis(isobutyronitrile)
AMP	- adenosine monophosphate
ATP	- adenosine triphosphate
BSA	- bis(trimethylsilyl) acetamide
BuLi	- butyl lithium
C.I.	- chemical ionisation
DAHP	- 3-dioxy-D-arabinoheptulosonic acid 7-phosphate
DAST	- diethylamino sulphur trifluoride
DBU	- 1,8-diazobicyclo[5-4-0] undec-7-ene
DCC	- dicyclohexylcarbodiimide
DCM	- dichloromethane
DCU	- dicyclohexylurea
ddA	- 2',3'-dideoxyadenosine
ddAMP	- 2',3'-dideoxyadenosine monophosphate
ddATP	- 2',3'-dideoxyadenosine triphosphate
ddI	- 2',3'-dideoxyinosine
DMAP	- 4-dimethylaminopyridine
DMF	- N,N-dimethylformamide
DMSO	- dimethylsulphoxide
DDVP	- dimethyl 2,2-dicholorovinylphosphate
D_2O	- deuterium oxide
EAAR	- excitatory amino acid receptor
E.I.	- electron ionisation
EPSP	- 5-enolpyruvylshikimate -3-phosphate
Et	- ethyl
FAB	- fast atom bombardment

FBPase	- fructose 1,6-bisphosphatase
h	- hour
IR	- infra-red
J	- coupling constant
KA	- agonists kainatic
LDA	- lithium diisopropylamine
MCPBA	- meta chloro perbenzoic acid
Me	- methyl
MGluR	- metabotropic glutamate receptor
m.p.	- melting point
ms	- mass spectroscopy
NAD(P)H	- nicotinamide adenine dinucleotide (phosphate), reduced form
$NAD(P)^+$	- nicotinamide adenine dinucleotide (phosphate),
NBA	- nitro benzyl alcohol
NMDA	- N-methyl-D-aspartate
NMR	- nuclear magnetic resonance
n.O.e	- nuclear Overhauser effect
PEP	- phosphoenolpyruvate
PI	- phosphoinositide
ppm	- parts per million
Rf	- retention time
r.t.	- room temperature
S3P	- shikimate 3-phosphate
t-ACPD	- trans-1-amino cyclopentane-1,3-dicarboxylic acid
Tf_2O	- triflate (trifluoromethane sulfonic anhydride)
TFA	- trifluoroacetic acid
TFPE-FTCM	- 2,2,2-trifluoro -1- phenyl ethanol fluorotrichoromethane
TG	- triglyme
THF	- tetrahydrofuran

TLC	- thin layer chromatography
TMSI	- iodotrimethylsilane
α-MBA	- α-methylbenzyl amine
URP	- under reduced pressure

PART I

ORGANOPHOSPHORUS COMPOUNDS

CHAPTER ONE

INTRODUCTION

PART I - ORGANOPHOSPHORUS COMPOUNDS

CHAPTER ONE - INTRODUCTION

1.1 Introduction

Organophosphorus compounds are important in the biochemical processes of all living systems which require orthophosphate (PO_4^{3-}) as a primary constituent of nucleic acids, phospholipids, phosphorylated proteins and carbohydrates. Organophosphate compounds also have phosphorylating and alkylating properties and, in certain cases, the alkylating property appears to be biologically important. The inhibition of protein biosynthesis by the alkylation of DNA is believed to be the primary effect of organophosphorus chemosterilants[1].

Biological oxidation is also very important in the metabolism of organophosphorus pesticides with respect to their activation and detoxication. Insecticidal activity and mammalian toxicity are generally accepted as due to phosphorylation of acetylcholinesterase. Besides acetylcholinesterase, organophosphorus compounds also inhibit cholinesterase and serine proteinases, by phosphorylating the serine hydroxyl group in the active site of the enzyme molecule[1].

Although all organic esters of phosphate are loosely classified as organophosphates, it is the phosphonate class which contains a carbon-phosphorus covalent bond that will be the main subject of this Thesis . The organophosphates which occur naturally in living systems are usually oxygen esters, diesters, or anhydrides of phosphoric acid. In 1959 Horiguchi and Kandatsu[2] first identified naturally-occurring phosphonates. Since then it has been shown that phosphonates occur naturally, in a variety of organisms, and several metabolic processes involving phosphonates have been elucidated. Phosphonates are known to occur only in a relatively few species.

Studies of the biosynthetic pathways leading to the phospholipid precursor, 2-aminoethyl phosphonate (1) in *Tetrahymena pyriformis*, and to the antibiotics, fosfomycin (2), and bialaphos (3) in certain strains of *Streptomyces*, suggest that phosphoenolpyruvate (PEP) is the key precursor (**Figure 1**) of naturally-occurring phosphonates [3].

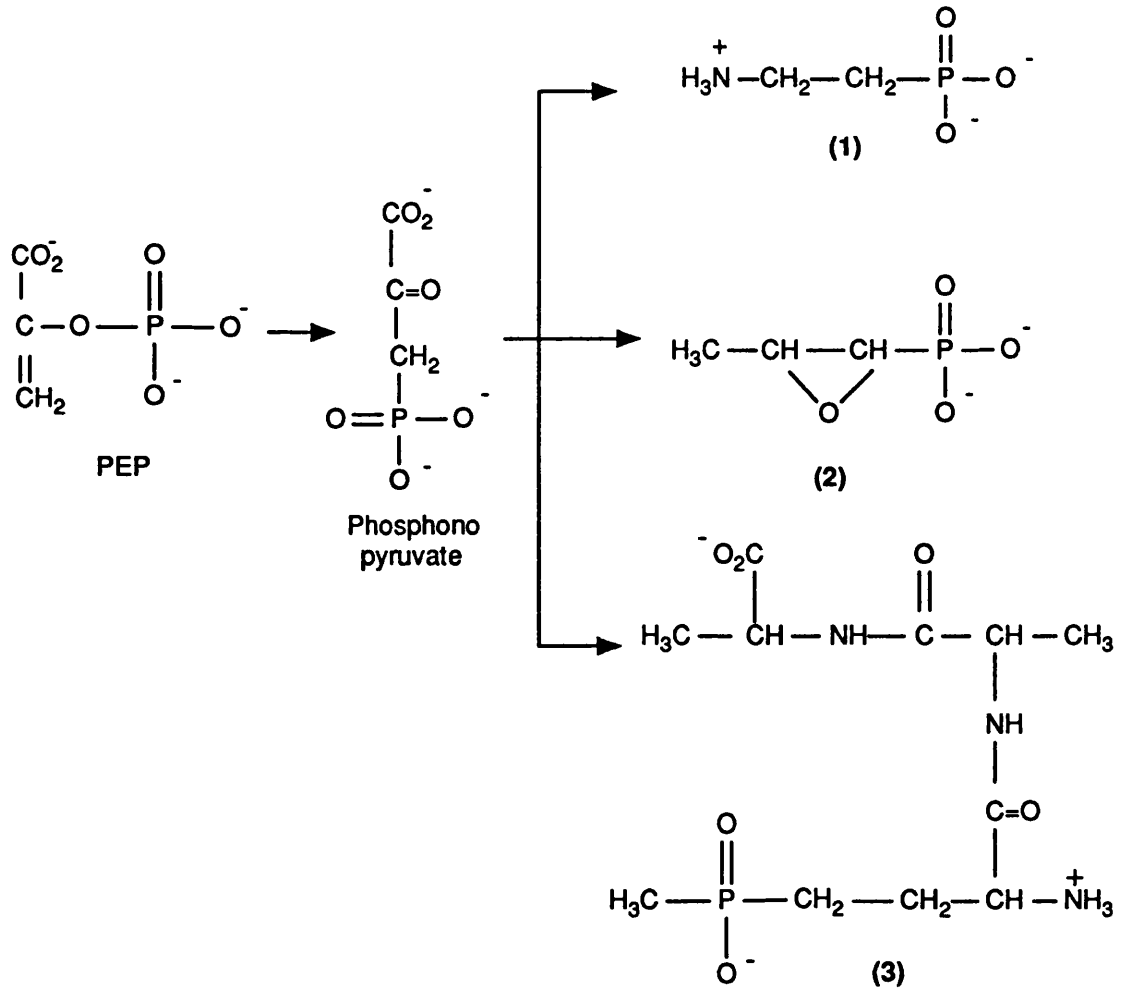


Figure 1 - PEP as a key precursor of naturally occurring phosphonates

The synthetic study of organophosphorus compounds was initiated by Lassaigne[4] in 1820 with the esterification of alcohols with phosphoric acid.

In 1845 Thénard prepared a series of phosphine derivatives[4]. Michaelis, then A. E. Arbuzov and, later his son B. A. Arbuzov, dominated the field for many years. Since then, the preparation of a majority of the numerous reported organic phosphonates has been accomplished by the general and versatile Michaelis-Arbuzov reaction[5], a very important and useful process which serves as a simple, one-step method, to directly introduce a carbon-phosphoryl bond into an organic compound.

1.2 Commercial Uses of Phosphonates

Commercial interest in phosphonates is increasing because of the recognition of their unique chemical properties. The types of phosphonates of potential interest include catalysts, lubricants, additives, flame retardants, surfactants, corrosion inhibitors, plasticizers, bonding agents for rocket fuel, and insecticides. Indeed, the single biggest-selling herbicide in the world, Roundup containing Glyphosate, is a phosphonate.

1.3 The Role of Phosphonates in Living Systems

Phosphorus plays a vital role in all life forms, and phosphonates are an important mode in which it performs its essential functions. The phosphonate class of synthetic organophosphorus molecules contains many compounds which are extremely reactive in biological systems.

Phosphonates have been observed to be resistant to enzymatic hydrolysis, and an example is the resistance of ceramide-AEP (2-aminoethyl phosphonic acid) to hydrolysis by phospholipase C from *Clostridium welchii*, an enzyme that ordinarily cleaves the phosphate ester bond between ethanolamine phosphate and sphingosine.

Another role which phosphonates play in organisms is directly related to the properties of the molecule itself. The inert character of phosphonate-containing molecules may be used to generate membranes, or structural components, which are resistant to oxidation, hydrolysis, or other metabolic processes. Whether this resistance is fortuitous, or whether there is an actual biological requirement, is not known. The specificity of incorporation of N-methyl-AEP into sphingolipids, noted by Matsura[2], is indicative of either a special function or a unique metabolic pathway for incorporation into that material. A number of authors have made proposals concerning the physiological function of the phosphonates' molecules, resulting in the provision of a buffering, or a cationic, binding capacity[2]. Rosenberg[2] proposed that in *T. pyriformis*, the AEP was incorporated into structural material and conferred resistance to enzymatic hydrolysis on the cell membrane. This AEP containing material is apparently not metabolized further, is necessary for structural integrity, and is synthesized only during cell growth.

Another class of phosphonate of biological significance is that of the phosphonate antibiotics. The first antibiotic to be isolated and characterized was phosphomycin, (-)(1R,2S)1,2-epoxypropylphosphonic acid (**Figure 2**), which is used in the inhibition of bacterial cell wall biosynthesis. More recently, several other antibiotics containing phosphonate have been isolated from various species of the genus *Streptomyces*.

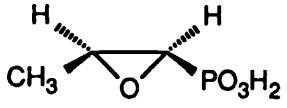


Figure 2 - Phosphomycin

The interest in phosphonic acids and their esters is increasing following the recognition of their possibilities in metabolic regulation and perturbation. As a substitute for a natural phosphate metabolite, a phosphonic acid or a phosphonate ester, may be capable of inhibiting, or perturbing, the regular metabolism of an organism simply by non-participation in a normal phosphate cleavage process. The metabolic alterations may be intended for therapeutic purposes to inhibit life processes. The phosphonic acid might also be capable of specific, or nonspecific, inhibition of one or more enzymatic processes.

Phosphonoacetic acid has been shown to supress replication of DNA tumor viruses by inhibiting the activity of virus-induced DNA polymerase and, consequently, viral DNA synthesis[6]. It has also been shown to inhibit effectively the replication of Herpes viruses[7]. H. S. Allaudeen *et al.*[6] showed that phosphonoacetic acid inhibits cellular DNA polymerase α, β and γ of L1210 cells as well as reverse transcriptase of two type C viruses.

S. F. Martin *et al.*[8] commented that the replacement of a phosphate functional group with a phosphonate moiety in biologically important molecules, constitutes an important process to develop non-hydrolyzable substrate analogues as inhibitors, or alternative substrates, for enzymes that process naturally-occurring phosphates, as shown is section 1.6.

As pesticides, herbicides, fungicides, rodenticides, and a number of other biocidal materials, the phosphonates are very useful in the control of biological species considered to be pests[1]. Dimethyl 1-hydroxy-2,2,2-trichloroethylphosphonate (trichlorfon)(**Figure 3**) is a pesticide with high insecticidal activity, particularly against *Diptera*. It is useful for the control of both sucking and chewing insects on field crops, vegetables, and seed crops. It is also useful for the control of insects of public health significance and animal ectoparasite because of its low toxicity.

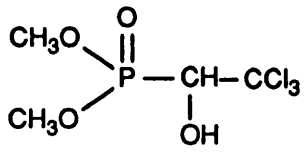


Figure 3 - Trichlorfon

The primary herbicide is glyphosate, N-[phosphonomethyl]glycine (**Figure 4**). This herbicide is non selective and is effective against various annual and biennial species of grass, sedges, and broad-leaved weeds.

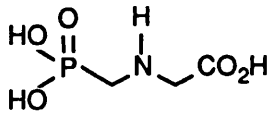


Figure 4 - Glyphosate

Phosphonomycin (**Figure 2**), which is produced by strains of *Streptomyces*, is in practical use in agricultural fungicides in Japan.

Gophacide, di-(*p*-chlorophenyl) N-acetimidophosphoramidothionate (**Figure 5**), although not formally a phosphonate, may be mentioned at this point, being one of the rare examples of organophosphorus compounds useful as a rodenticide. Gophacide is not active as an anticholinesterase *in vitro*, but is slowly activated *in vivo*. The slow onset of symptoms may be responsible for the acceptability of this organophosphorus compound to rodents as a constituent of baits, because it makes it possible for the rodent to consume a lethal dose[2].

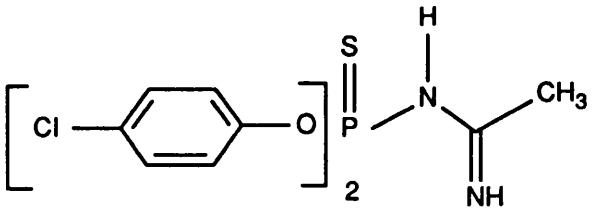


Figure 5 - Gophacide

The utility of organophosphonates is being widely exploited, and they are expected to have potential applications as phosphate mimetics.

1.4 Phosphoenolpyruvate (PEP)

The phosphorylated compounds found in cells are often classified as high-energy or low-energy according to the magnitude of the $\Delta G^{o'}$(free energy change) value for their hydrolysis.

Phosphoenolpyruvate is well known as one of the high-energy phosphate compounds for which $\Delta G^{o'}$ of hydrolysis is highly negative and which, therefore, can serve as phosphate donors to ADP (adenosine diphosphate), playing a vital role in ATP synthesis.

ATP (adenosine triphosphate) is the principal high energy intermediate or carrier compound in the living cell (**Figure 6**).

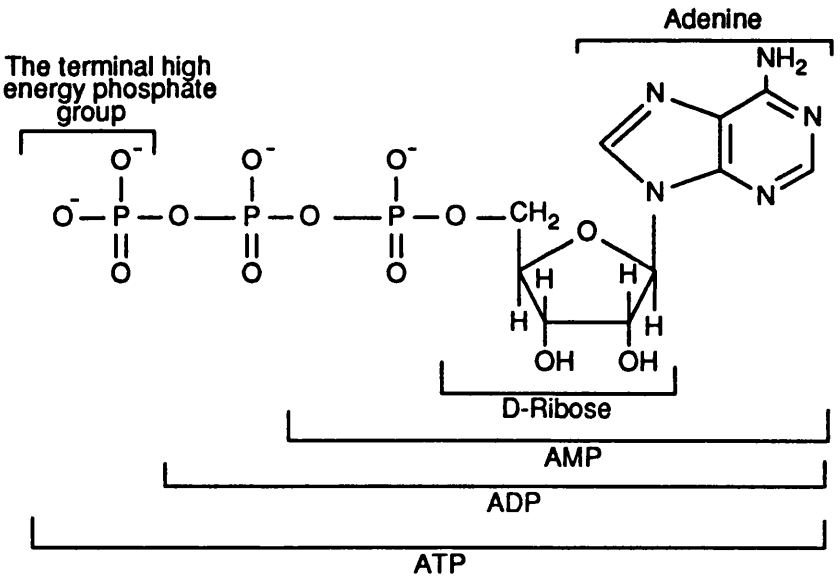


Figure 6- Adenosine Triphosphate

ATP and its successive hydrolysis products, adenosine diphosphate (ADP) and adenosine monophosphate (AMP), are nucleotides [9]. Nucleotides consist of a heterocyclic purine or pyrimidine base, a 5-carbon sugar, and one or more phosphate groups. In ATP, ADP, and AMP the base is the purine adenine, and the 5-carbon sugar is D-ribose. Many nucleotides are known, differing in their sugars and nitrogenous bases. Nucleotides have a variety of cell functions, but are particularly well known as building blocks of DNA and RNA, in which they function as coding elements. ATP, ADP and AMP are present in all forms of life and serve the same universal functions. They occur not only in the cell cytosol but also in mitochondria and the cell nucleus.

ATP functions as an energy - carrying common intermediate in the cell, linking the reactions delivering free energy and those requiring it. During energy-yielding catabolic reactions super high-energy phosphate compounds are generated at the expense of energy released on degradation of organic cell nutrients. A specific enzyme, called a kinase, then catalyzes the transfer of a phosphate group from such a super high-energy phosphate compound, designated X—Ⓟ , to ADP to form ATP. In the second step another specific kinase catalyzes the transfer of the terminal phosphate

group from ATP to an acceptor molecule, say Y, whose energy content is increased when it accepts the phosphate to become $Y—Ⓟ$. These two reactions can be written:

$$X—Ⓟ + ADP \longrightarrow X + ATP$$

$$ATP + Y \longrightarrow ADP + Y—Ⓟ$$

The net effect of these two reactions, coupled by the common intermediate ATP, is the transfer of chemical energy from $X—Ⓟ$ to Y via transfer of the phosphate group. ATP is nearly always the mediator of such phosphate group transfer reactions, since cells generally do not contain kinases that can transfer phosphate groups directly from super high-energy phosphate compounds to low-energy acceptors.

Two important donors of phosphate groups to ADP are the compounds 3-phosphoglyceroyl phosphate and phosphoenolpyruvate. Both are formed during the energy-yielding degradation of glucose to yield lactate, a process called glycolysis (**Figure 7**).

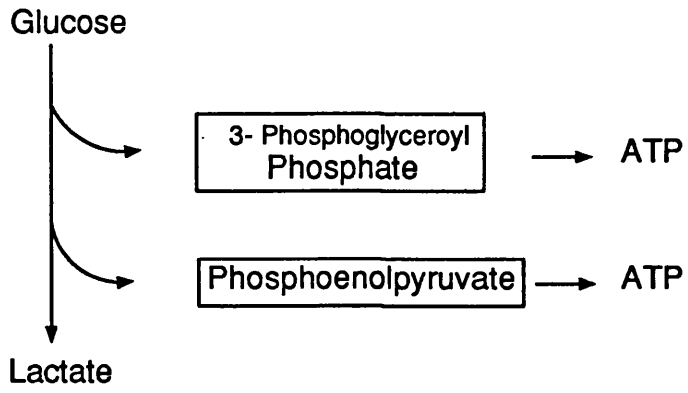
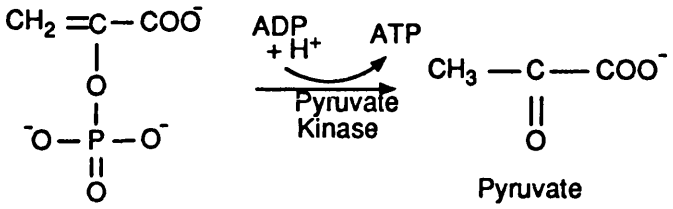


Figure 7 - The process of glycolysis

A large part of the free energy release on degradation of glucose to lactate is conserved in these two compounds. In the cell these high-energy phosphate compounds do not undergo hydrolysis; instead their phosphate groups are transferred to ADP to yield ATP by the action of specific kinases.

Phosphoenolpyruvate donates its phosphate group to ADP in a reaction catalysed by pyruvate kinase.



This reaction of phosphoenolpyruvate tends to go far to the right under standard conditions, because $\Delta G^{o'}$ for hydrolysis of phosphoenoylpyruvate (-14.8 Kcal/mol) is much larger than $\Delta G^{o'}$ for hydrolysis of ATP.

The three aromatic amino acids phenylalanine, tyrosine, and tryptophan are formed from phosphoenolpyruvate[9]. In the first step, phosphoenolpyruvate (**5**) reacts with erythrose-4-phosphate (**6**) to produce the seven-carbon saccharide 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP, **7**)). After cyclization and dehydration, the product is reduced to shikimic acid (**4**), an important intermediate in the synthesis not only of these three amino acids, but also of many other aromatic compounds (particularly in plants), (**Figure 8**)

Shikimic acid is phosphorylated by shikimate kinase and ATP, and then reacts with a further molecule of phophoenolpyruvate to yield chorismic acid (**12** in **Figure 8**), which is the base compound in the common aromatic sequence. This metabolite route from glucose to chorismic acid is generally referred to as the common pathway (**Figure 8**).

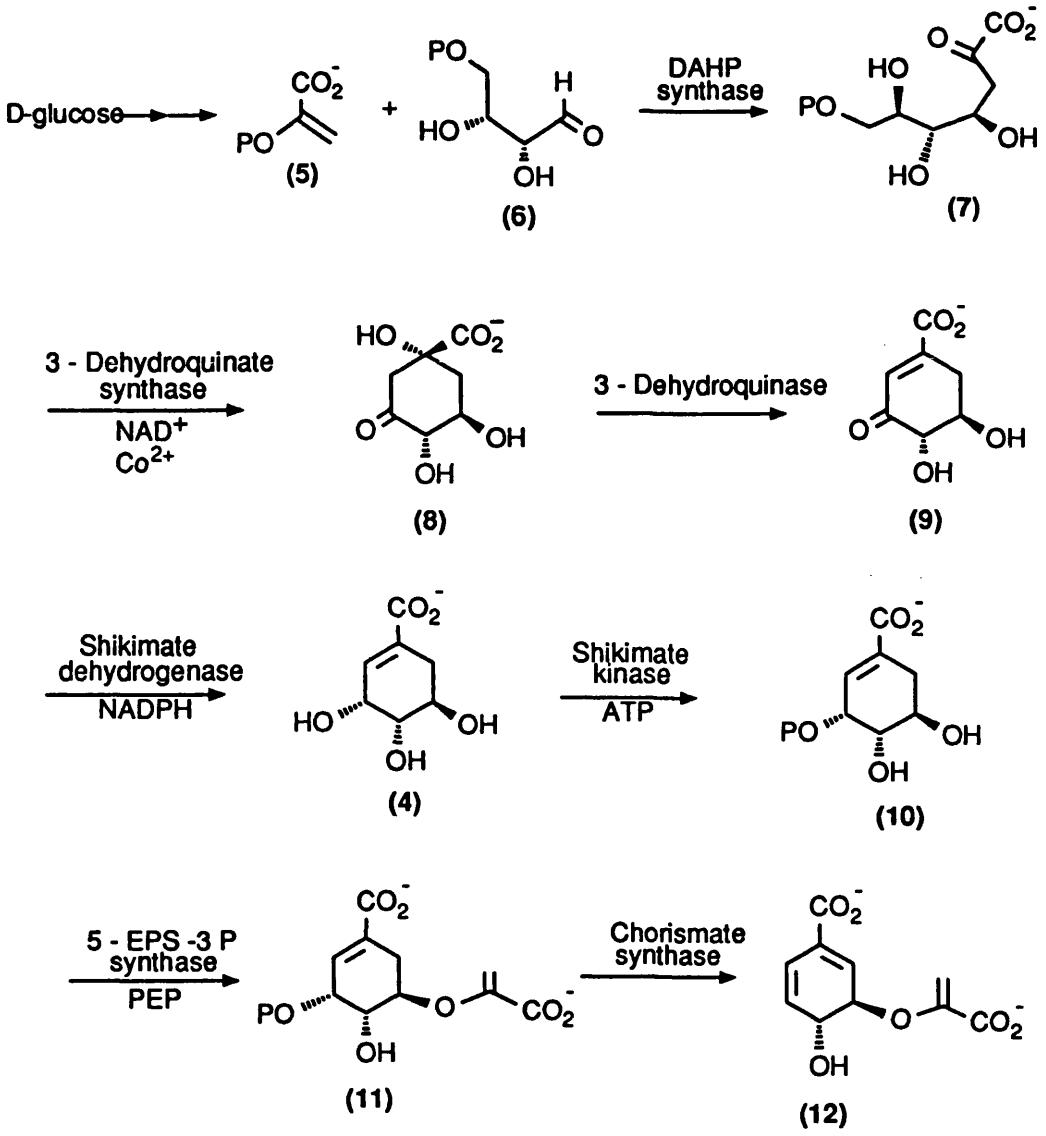


Figure 8 - The Common Pathway

In the synthesis of phenylalanine and tyrosine, chorismate is rearranged to form prephenate, and then decarboxylated and reduced. The resulting phenylpyruvate is transaminated to yield phenylalanine, which can be hydroxylated to tyrosine (**Figure 9**).

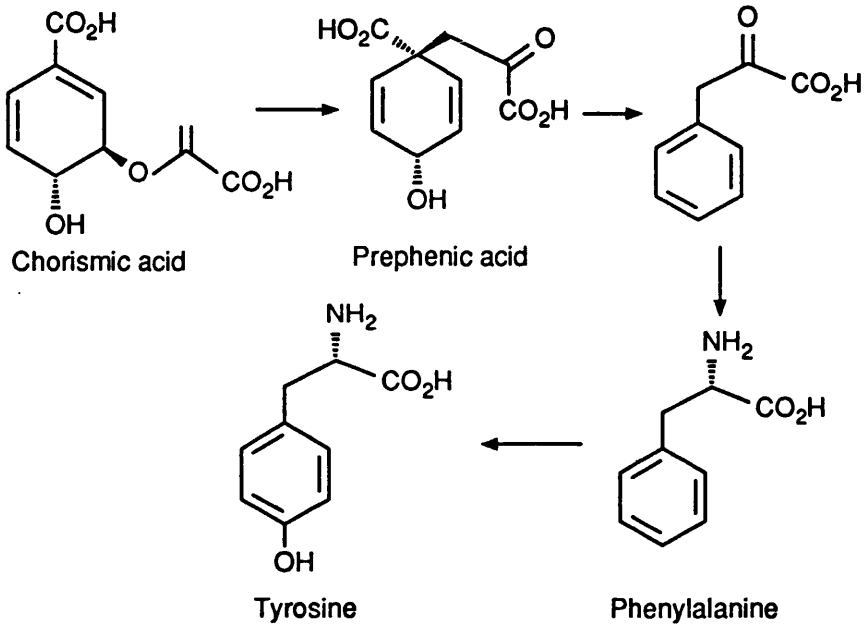


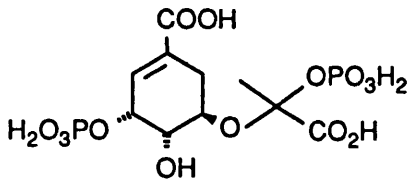
Figure 9 - Synthesis of phenylalanine and tyrosine

The shikimate pathway is present in all plants and microorganisms, but is completely absent in mammals, birds, and insects[10]. The three aromatic amino acids, therefore, cannot be produced by *de novo* synthesis in animals, but must be obtained from the diet.

The shikimic acid pathway is thus vital for the metabolism of plants and lower organisms, but does not operate in animals. This may allow the development of herbicides, and selective fungicides or bactericides, which are non-toxic to animals.

5-Enolpyruvyl shikimate-3-phosphate synthase (5-EPS-3P synthase) is an important enzyme in the shikimate pathway that catalyzes the reversible transfer of the carboxyvinyl moiety of phosphoenolpyruvate (PEP) to the hydroxyl group of carbon 5

of shikimate 3-phosphate (S3P), (**10**), with the formation of a tetrahedral intermediate (**13**), followed by elimination of the phosphate, to produce 5-enolpyruvylshikimate-3-phosphate (5-EPS-3-P)(**11**)[11]. From a commercial viewpoint, it is the most important enzyme in the shikimic acid pathway since it is inhibited by glyphosate, (N-phosphonomethyl glycine) (**Figure 4**), the active ingredient of the herbicide Roundup[12].



(13)

The shikimic acid pathway commences with erythose-4-phosphate (formed in the photosynthetic carbon cycle) and phosphoenolpyruvate (formed in the glycolytic pathway) and passes, via shikimic acid-3-phosphate, to chorismate. Thereafter the path branches to tryptophan (via anthranilate) and to phenylalanine/tyrosine (via prephenate). The glyphosate is a specific inhibitor of one enzyme in that pathway, namely the synthase that catalyses the conversion of shikimic acid-3-phosphate to the 3-phosphate derivative of 5-enolpyruvyl shikimate (**Figure 10**)[13]. Amrhein *et. al*[14] demonstrated that the action of glyphosate resulted in a massive accumulation of shikimic acid and a shortage of phenylalanine, leading to a decrease in the rate of protein synthesis.

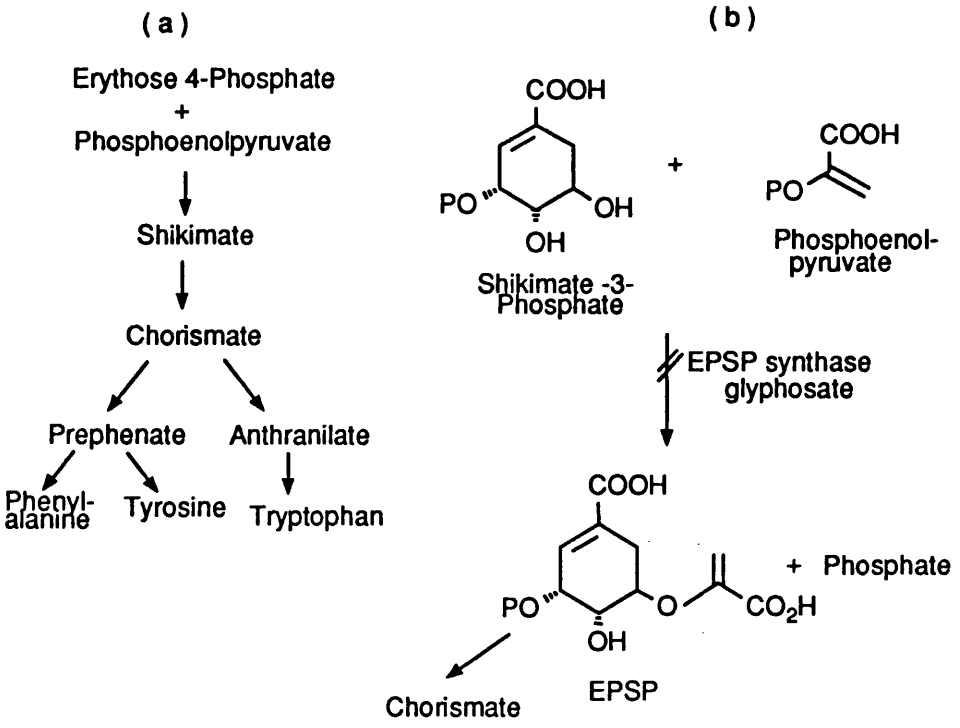
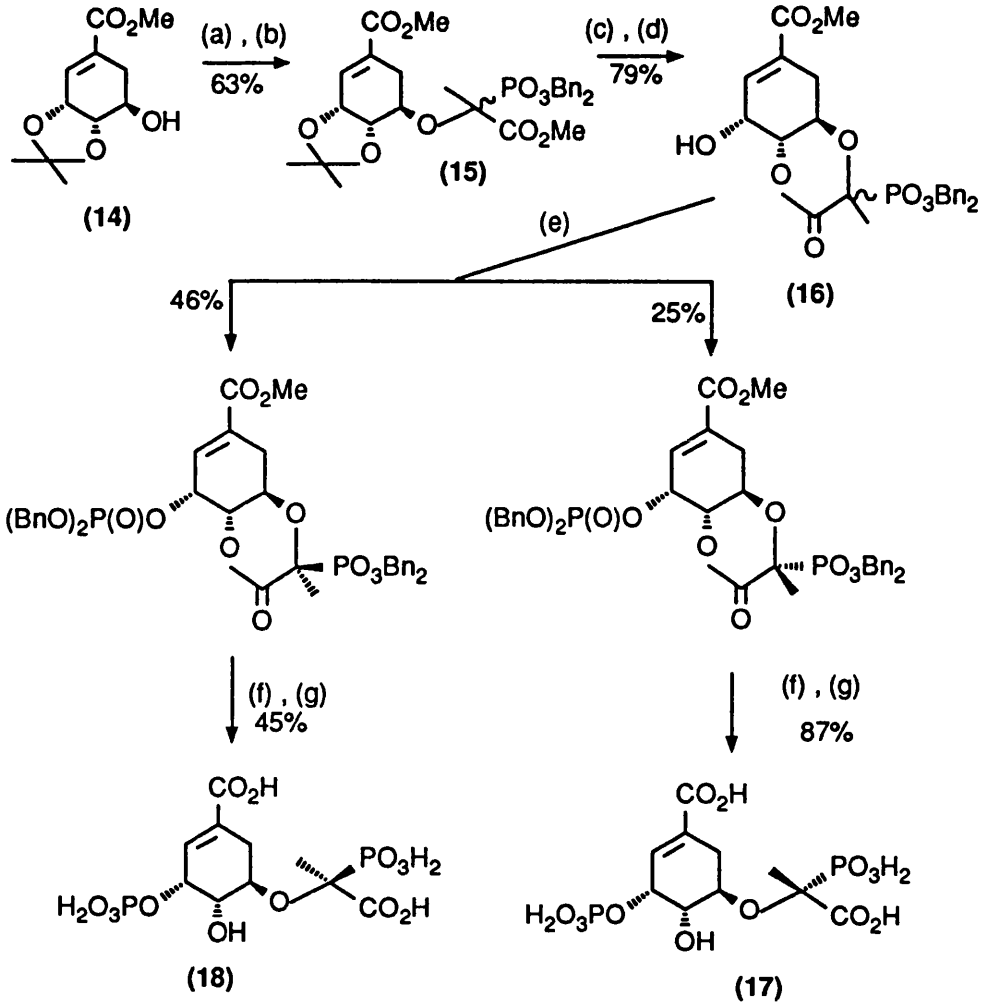


Figure 10 - (a) Outline of the shikimic acid pathway; (b) the step in the pathway that appears to be most vulnerable to glyphosate.

Of all enzymes involved in the shikimate pathway, only 5-enolpyruvylshikimic acid-3-phosphate synthase is inhibited by the glyphosate [14].

Bartlett *et al*[11] have synthesized a number of analogues of the unstable tetrahedral intermediate (**13**) involved in the 5-EPS-3-P reaction. Two strategies were chosen in order to stabilise the ketal phosphate structure of the tetrahedral intermediate (**13**). In the first, the phosphate was replaced by a phosphonate moiety. The phosphonates were synthesized from the acetonide of (-)-methyl shikimate (**14**)(**Scheme 1**). Rhodium diacetate coupling of (**14**) with methyl(dibenzylphosphono) diazoacetate, followed by methylation, afforded the diastereomeric phosphonates (**15**). Deprotection and cyclisation gave the lactones (**16**), which were phosphorylated prior to chromatographic separation. Deprotection of both diastereomers yielded the phosphonate analogues (**17**) and (**18**).

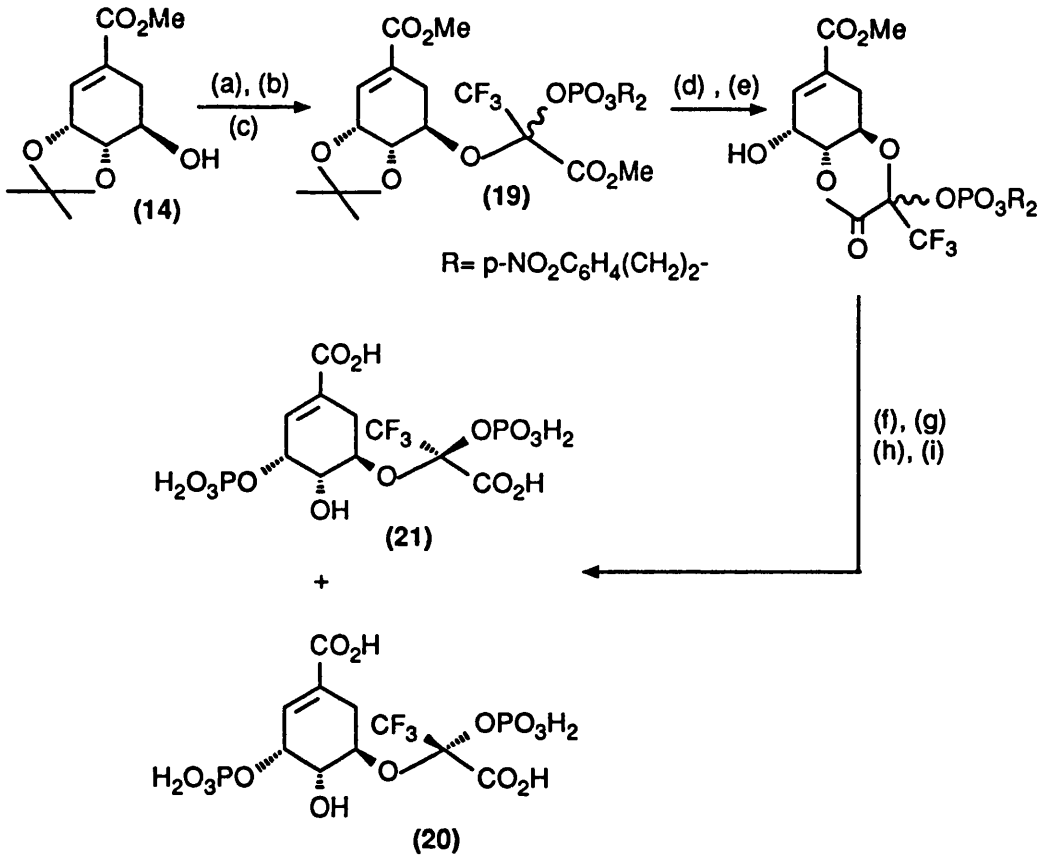


Scheme 1

Reagents and conditions: (a) $(BnO)_2P(O)C(N_2)CO_2Me$, $Rh_2(OAc)_4$, PhH, reflux; (b) KH, MeI, THF; (c) p-TSA, aq.MeCN; (d) p-TSA, PhH, reflux; (e) LDA, $[(BnO)_2P(O)]_2O$, THF, -78°C; (f) TMSBr; (g) aq. NaOH

In the second, electron withdrawing substituents were introduced into the methyl group of compound (**13**) in order to destabilise the oxacarbonium ion presumably involved in the elimination process. The trifluoropyruvate phosphate analogues were synthesized from the acetonide of (-)-methyl shikimate (**14**)(**Scheme 2**). Reaction of (**14**) with methyl trifluoropyruvate gave the hemiketal, which was

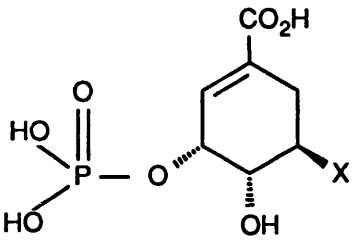
phosphorylated to afford the diastereomeric phosphates (**19**). Lactone formation, further phosphorylation and deprotection, yielded the trifluromethyl analogues (**20**) and (**21**).



Scheme 2

Reagents and conditions: (a) $CF_3C(O)CO_2Me$, PCl_3;
(b) p-$NO_2C_6H_4(CH_2)_2OH$; (c) m-CPBA; (d) H_3O^+; (e) K_2CO_3;
(f) $[NO_2C_6H_4(CH_2)_2O]_2PN(i\text{-}Pr)_2$; (g) m-CPBA; (h) DBU, BSA;
(i) aq. NaOH

Recently Anderson *et al*[10] prepared two new inhibitors of 5-EPS-3-P synthase (**22** and **23**) to probe the shikimate 3-phosphate binding site.



(**22**) X = H

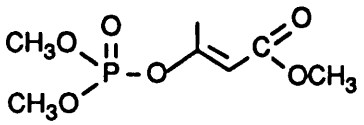
(**23**) X = NH_2

Phosphorus, in the form of phosphate esters, particularly ADP and ATP, plays a vital role in the chemistry of life, but there are some phosphorus compounds that are also vehicles of death, being used as pesticides and chemical warfare agents. In the course of the evaluation of thousands of phosphorus compounds for their insecticidal qualities, enol phosphates have attracted considerable attention. The insecticidal properties and merits of many enolphosphates have been reported[4], and there are three commercially important systems: Phosdrin, DDVP, and Phosphamidon (**Figure 11**).

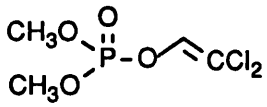
Phosdrin has been applied to plants for the economic control of insects. It is absorbed shortly after application, and then rapidly translocated throughout the plant.

Phosphamidon is an analogue of Phosdrin in which the ester group is replaced by an amide. Phosphamidon has quite promising insecticidal qualities.

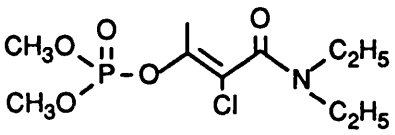
The insecticidal behavior of DDVP (dimethyl 2,2-dichlorovinyl phosphate) has been thoroughly investigated and it is effective against houseflies, cigarette beetles, and for pest control in tobacco warehouses.



Phosdrin



DDVP



Phosphamidon

Figure 11 - Structures of Phosdrin, DDVP and Phosphamidon

1.5 Phosphonate Analogues

It is well known that analogues of natural products frequently inhibit enzymes, and that this effect is often highly specific.

Inhibitors are divided into two major classes: irreversible and reversible[15]. An irreversible inhibitor reacts covalently with an enzyme preventing substrate binding or catalysis. A reversible inhibitor undergoes rapid equilibrium binding with the enzyme. Reversible inhibitors may be further classified as competitive, uncompetitive, or non-competitive, depending on whether they bind to the free enzyme, to the enzyme-substrate complex, or both, respectively.

Phosphonates have been widely investigated as models for phosphate esters because they have an isosteric relationship, and because the C-C-P linkage is more stable to hydrolysis than is the C-O-P linkage[16]. Phosphonates might be expected to act either as substrates or as competitive inhibitors of enzymes that catalyze reactions of the corresponding phosphate esters[17].

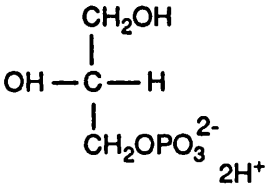
Several changes in properties must be considered in substituting a phosphonic acid for a phosphate, aside from the stability of the phosphonic acids to enzymatic hydrolysis by normal routes[18].

Oxygen and carbon have substantially different electronegativities (3.5 and 2.8 respectively) which results in an altered electron distribution between phosphates and phosphonates. The introduction of the electron donating alkyl group thus decreases the acidity of the phosphorus-containing acid function.

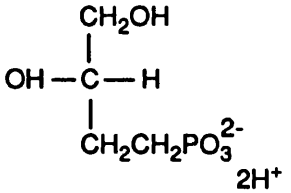
A second factor of change is that of physical size and shape. Simply replacing the phosphate ($-OPO_3H_2$) with a phosphonic acid ($-PO_3H_2$) group, obviously contracts the overall size. More specifically, the distances between the phosphoryl oxygen and the other possible points of interaction on the molecule (for example, hydroxyl groups on a carbohydrate ring), are significantly changed[19].

For this reason Engel[20] suggested that the isosteric phosphonic acids, in which the O atom of the phosphate linkage has been replaced by CH_2 group, may be considered as better analogues.

Adams *et al.*[21] synthesized S-(+)-3,4-dihydroxybutyl phosphonic acid, an isosteric analogue of *sn*-glycerol 3-phosphate (**Figure 12**), that behaved as an effective substrate for glycerol 3-phosphate dehydrogenase of rabbit muscle, showing kinetic parameters very similar to those obtained with the natural substrate.



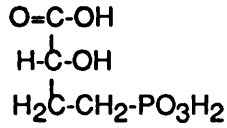
sn - glycerol 3-phosphate



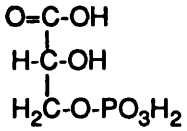
S-(+) -3,4- dihydroxybutyl phosphonic acid

Figure 12 - Structures of S-(+) -3,4- dihydroxybutyl phosphonic acid and *sn* - glycerol 3-phosphate

Dixon *et al.*[22] prepared 2-hydroxy-4-phosphonobutyric acid, the analogue of 3-phosphoglyceric acid (**Figure 13**), in which the $-O-PO_3H_2$ group is replaced by $-CH_2-PO_3H_2$. This analogue is capable of exerting an inhibitory effect on cells containing an active glycerol-3-phosphate dehydrogenase, and it is also a substrate for rabbit muscle glycerol-3-phosphate dehydrogenase[23].



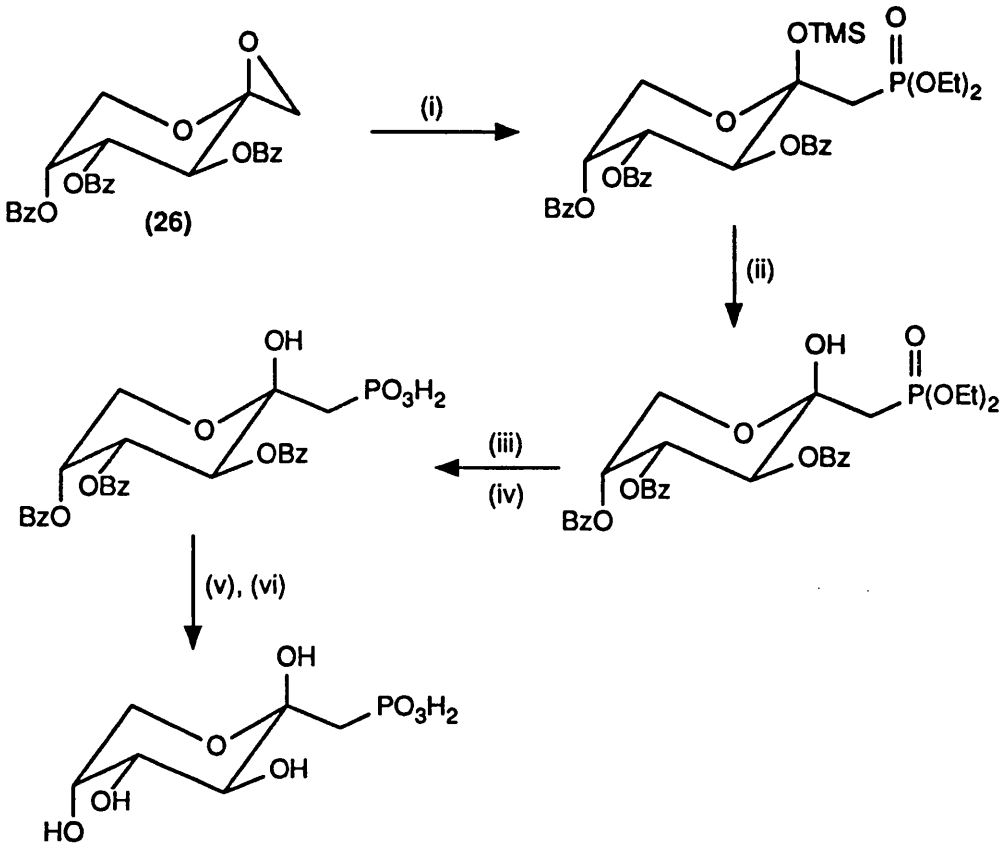
2-hydroxy-4-phosphono butyric acid



3-phosphoglyceric acid

Figure 13 - Structures of 2-hydroxy-4-phophono butyric acid and 3-phosphoglyceric acid

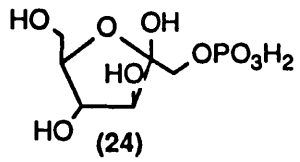
The synthesis of a phosphonate analogue of fructose phosphate, in which the phosphate group was directly replaced by the phosphonate moiety, was developed at the University of Bath, by Campbell *et al.*[19]. The authors described the synthesis of D-fructose 1-deoxy 1-phosphonic acid (**25, Figure 14**), an analogue of D-fructose 1-phosphate (**24, Figure 14**), utilising the reaction of diethyl trimethylsilyl phosphite with a novel spiro anomeric epoxide (**26**), derived from fructose (**Scheme 4**).



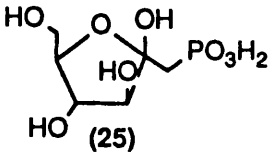
Scheme 4 Reagents and conditions:

(i) $(EtO)_2P(O)TMS$, 90°C, 4h; (ii) Bu_4NF, THF, 0°C, 10 min (78%); (iii) TMSBr, CH_2Cl_2, r.t., 24h; (iv) H_2O, r.t., 2h (98%); (v) NaOH, MeOH, r.t., 2h, Dowex 50(H^+); (vi) $(C_6H_{11})_2NH$, MeOH, acetone (22%).

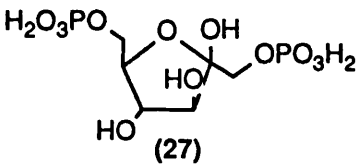
The phosphate ester of D-fructose plays an essential role in the regulation of glycolysis and gluconeogenesis. The enzyme involved in gluconeogenesis is fructose 1,6-bisphosphatase (FBPase) which catalyses the hydrolysis of fructose 1,6-bisphosphate (**27, Figure 14**) to fructose 6-phosphate (**28, Figure 14**). As the control element of gluconeogenesis, FBPase plays an essential role in controlling blood sugar levels. If the 1-phosphate group is replaced by a non-hydrolysable phosphonate group, the resulting molecule may still be recognised as a substrate by FBPase. This analogue would then act as a competitive inhibitor for FBPase by occupation of the active site.



(24)



(25)



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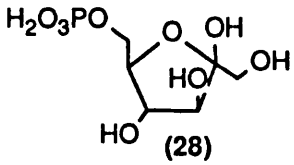
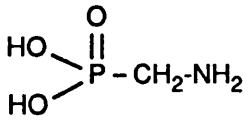


(28)

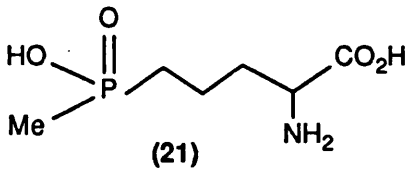
Figure 14 - Structures of D-fructose 1-phosphate (**24**), D-fructose 1-deoxy 1-phosphonic acid (**25**), fructose 1,6-bisphosphate (**27**), and fructose 6-phosphate (**28**)

1.6 Phosphoserine

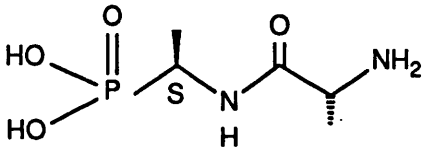
The study of phosphorus analogues of the natural α-amino acids that Chavane began in the 1940's[24], has accelerated in the past 10 years due to the discovery of molecules with useful biological activity. For example, the glycine analogue (**20**), is a plant growth regulant, the naturally occurring glutamic acid analogue (**21**) is a herbicide, and the dipeptide analogue (**22**) is an antibacterial agent that inhibits bacterial cell wall biosynthesis[24].



(20)



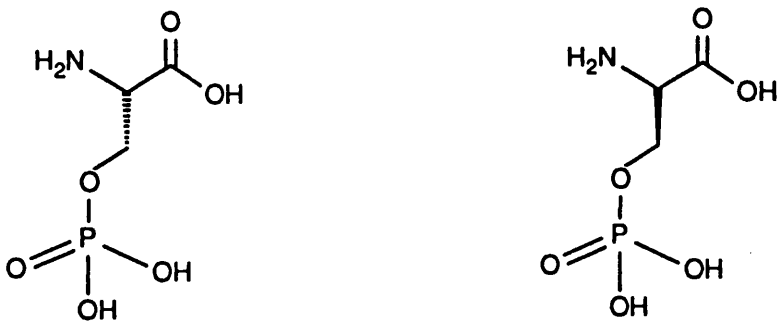
(21)



(22)

Serine phosphate is unique among the amino acid phosphates, for it occurs both in the membrane proteins and the membrane phopholipid and phophatidylserine[25]. It has been suggested that the ubiquity of serine phosphate is probably a result of its β-hydroxylamine chain, N-C-C-O , which is a common structural feature of the major phospholipids, sphingolipids, anaesthetics, cholinergists and other nerve and brain amines[25] .

Phosphoserine phosphatase is an enzyme that has an important metabolic role by virtue of its being a regulatory enzyme in serine biosynthesis, which in turn affects several metabolic pathways of vital importance, including lipid (sphingomyelin), neurotransmitter (glycine), and nucleic acid (purines) synthesis[26]. The enzyme was found to act on both L and D enantiomers of phosphoserine but with different degrees of affinity. The L-phosphoserine (**Figure 15**) was found to have a higher affinity with a Km value of 3.6×10^{-5}M as compared to D-phosphoserine (**Figure 15**) with a Km value of 1×10^{-4}M. Enzyme activity was found to be specific for phosphoserine, whereas other phosphoesters, including phosphothreonine and phosphoproteins such as casein and phosvitin, were found to be poor substrates. The enzyme activity was inhibited by vanadate (41%) at an equimolar concentration of 1mM. The two phenothiazine derivatives (trifluoperazine and chlorpromazine), and antiepileptics such as phenytoin and phenobarbital, were found to inhibit the enzyme activity under *in vitro* conditions. On the other hand diazepam, chlordiazepoxide and sucrose were found to have an activating effect on phosphoserine phosphatase.



L - Phosphoserine D - Phosphoserine

Figure 15 - Structures of L-phosphoserine and D-phosphoserine

L-Phosphoserine is a phosphomonoester which can be endogenously generated in the brain through various metabolic pathways, including breakdown of phospholipids following activation of phospholipase C. L-Phosphoserine has been reported to antagonise phosphoinositide (PI) hydrolysis in slices of rat brain as stimulated by agonists of the metabotropic glutamate receptor (mGluR)[27].

Data presented by Ichihara and Greenberg[28]indicated that phosphoserine is an intermediate in the biosynthesis of serine from carbohydrate precursors in rat liver. Similar observations have been made by Smith and Gunsalus[29] with cell-free extracts of *Escherichia Coli.*

Phosphoserine is present in the intermediate filament sub-units obtained from a variety of tissues and cells. Intermediate filaments, microfilaments, and microtubules and their associated proteins constitute the three major classes of cytoskeletal proteins of eukaryote cells. Of these three classes, the intermediate filaments of different cells are the least conserved in terms of their sub-unit complexity and properties[30]. Considerable morphological, immunological, and biochemical data suggests that intermediate filaments function in cells in coordination with other cytoskeletal proteins in many fundamental cellular activities, including protein synthesis, intracellular

organelle support and movement , and cellular division and locomotion. It is possible that much of this functional diversity is mediated by the complexity of the intermediate filament component.

Evidence accumulated in several studies indicates that the intermediate filaments of a variety of tissues and cell types are phosphorylated, presumably by cyclic nucleotide dependent protein kinases. In view of the important role of such kinases in the regulation of cellular activity, it seems possible that the phosphorylation of the intermediate filaments may, directly or indirectly, modulate their function in cells. Steinert *et al.*[30] confirmed that the sub-units of intermediate filaments of a variety of tissues and cell types contain phosphate, bound mostly as phosphoserine.

D-Phosphoserine was used to characterize the synaptic receptor of the inner retina. Excitatory neurotransmission in the central nervous system is largely mediated by acidic amino acid receptors that are, presumably, activated by glutamate, aspartate, or a closely related analogue. In the retina, all amacrine and ganglion cells are depolarized by the excitatory amino acid receptor (EAAR), agonists kainate (KA), N-methyl-D-aspartate (NMDA), and quisqualate (QQ).

To better characterize the synaptic receptors of the inner retina, a number of EAAR antagonists were surveyed to identify compounds that both reduced light-evoked excitatory postsynaptic potentials (EPSPs), and attenuated the depolarizing effects of exogenously applied KA, but not NMDA or QQ. Of the compounds that met these criteria the most interesting was D-phosphoserine, primarily because of its well-documented network-selective action in the retina.

The studies by Coleman *et al.*[31] showed that D-phosphoserine reduced the light-evoked excitatory postsynaptic potentials (EPSPs) of amacrime and ganglion cells. To determine which EAAR was blocked by D-phosphoserine, its antagonist action was challenged by coapplications of the different EAAR agonists. D-phosphoserine significantly reduced the depolarizing effect produced by KA, with little effect on NMDA and QQ responses, indicating that D-phosphoserine acted as a

reasonably selective KA-receptor antagonist.

The synthesis of phosphoserine peptides and their analogues is currently a subject of considerable interest due to the importance of protein phosphorylation in biology. Phosphorylation of proteins has been shown to be the main regulatory mechanism by which eukaryotic cells can convert neuronal or hormonal stimuli into intracellular responses[32]. In most instances, phosphate is bound as phosphoserine or phosphothreonine, while the phosphorylation of tyrosine may be involved in the mechanism of action of retroviral oncogenes and epithelial growth factor. Almost all work on protein phosphorylation has been carried out by studying the incorporation of radioactively labeled phosphate either *in vitro* or, in response to a provoked stimulus (hormone, growth factor, virus transformation, etc) *in vivo*.

Many biologically important proteins such as enzymes, growth factor receptors, cytoskeletal and contractile proteins, proteins acting in the cellular cycle, and oncogenic proteins are known to exist as phosphoproteins[33]. In addition, major proteins in bone, teeth, eggs and milk are also highly phosphorylated. Synthetic phosphopeptides related to biological phosphoproteins are increasingly used as models to study various aspects of protein structure and function[33].

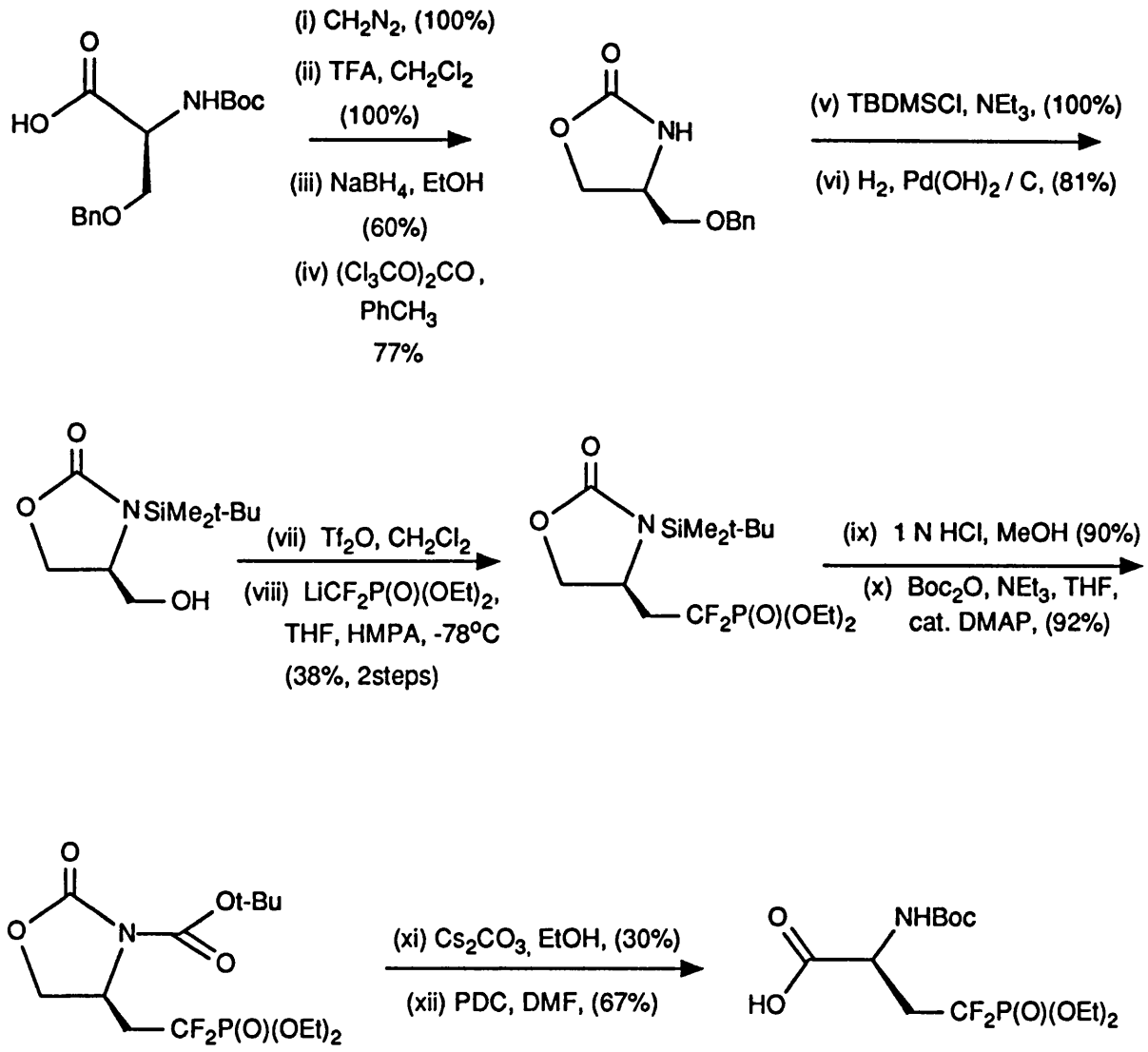
Phophorylation of ribosomal proteins was studied by L. Bitte and D. Kabat[34] in mouse sarcoma 180 tumor cells incubated in a nutrient medium with [^{32}P] orthophosphate. Ribosomal proteins are phosphorylated to form phosphoserine and phosphothreonine residues. The pattern of ribosomal protein phosphorylation in mouse sarcoma 180 cells is strikingly similar to that in rabbit reticulocytes. This similarity could prove that the phosphoproteins are true ribosome constituents rather than contaminants.

Ribosomes are complex organelles which contain many constituent proteins. They play a central role in biological information transfer. The proteins of eukaryote ribosomes are generally isolated after denaturation and cannot be assayed since they lack demonstrable biological activity. Consequently, it has not previously been possible

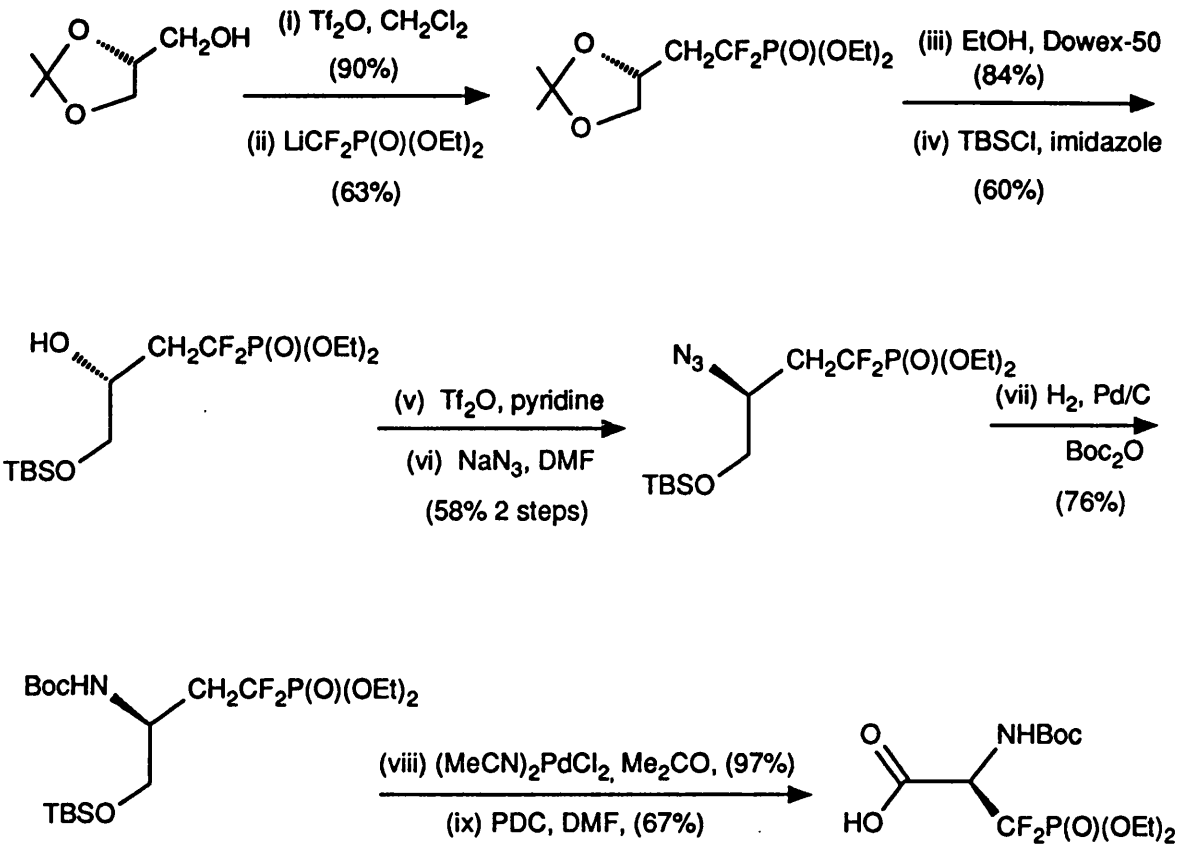
to show homology between ribosomal proteins from different mammalian species. However, the studies of L. Bitte and D. Kabat[34] showed that the homologous ribosomal phosphoproteins are well suited for further evolutionary studies because they can be readily identified and assayed. The study of the amino acid sequence of these phosphoproteins would help understanding of the evolution and function of ribosomes.

In a study of the synthetic processes of the Ehrlich ascites tumor in mice, E. P. Kennedy *et al.*[35] found that the phosphoprotein had a specific activity about 30 times higher than that of the phospholipid or nucleic acid fractions. Upon partial acid hydrolysis of the phosphoprotein, the authors isolated a phosphoserine of very high specific activity by chromatographic techniques.

The members of the protein phosphoserine/threonine phosphatase class of enzymes, PP1, PP2A, PP2B and PP2C, are important mediators of signal transduction events. For example, PP2B (calcineurin) is the common target of the immunosuppressants cyclosporin and FK-506. Enzymes in this family are known to dephosphorylate peptides, in addition to their usual protein substrates. Therefore, peptides containing an effective, and hydrolytically stable, phosphoserine mimic, are potential inhibitors of this class of enzymes[36]. Berkowitz *et al.*[36] synthesized the (α,α-difluoroalkyl) phosphonate analogue of L- phosphoserine in a form appropriate for solid phase peptide synthesis. Two independent routes were described, starting from L-serine or (R)-isopropylidineglycerol. In each case, PCF_2-C bond formation is achieved by triflate displacement with diethyl lithiodifluromethyl phosphonate (**Scheme 5** and **6**).

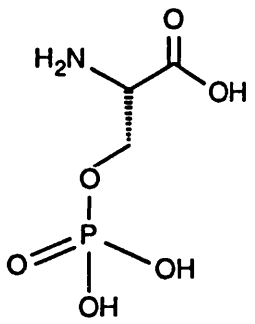


Scheme 5 - Route 1 for the synthesis of the α,α- (difluoroalkyl) phosphonate

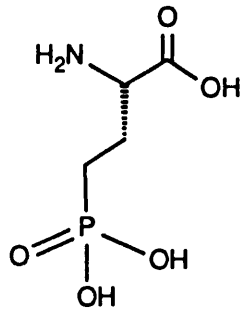


Scheme 6 - Route 2 for the synthesis of (α,α-difluroalkyl) phosphonate

While there have been numerous reports of the synthesis of phosphoserine, Ser(P) containing peptides there are, according to Shapiro *et al.*[37], few reports on the synthesis of peptides containing the corresponding phosphonate analogue, 2-amino-4-phosphono butanoic acid (Abu(P)) (**Figure 16**).



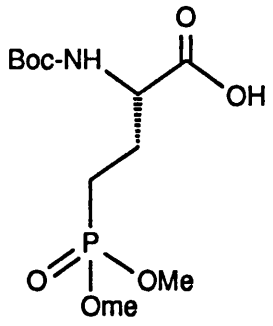
L - Phosphoserine



L - 2 - Amino - 4 - phosphono butanoic acid Abu (P)

Figure 16 - Structures of L-phosphoserine and Abu(P)

Such phosphoserine-peptides are clearly of interest since they would be stable to metabolism by protein phosphatases, and could be used to delineate biological mechanisms. Perich *et al.*[38] have reported to the solution phase synthesis of the tripeptide, H-Leu-Abu(P)-Glu-OH, using t-Boc strategy and the building block (**23**).



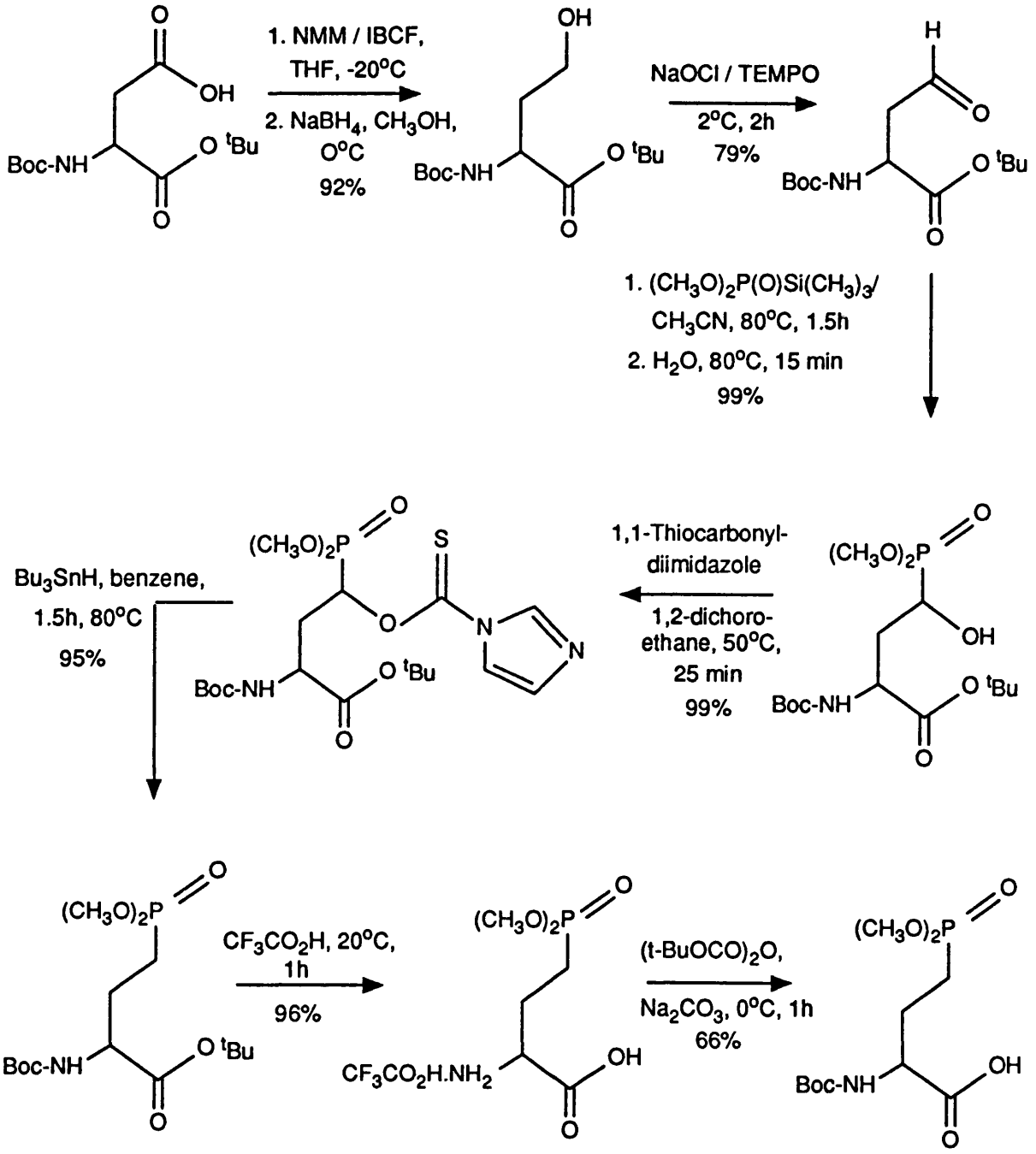
(23) Boc-Abu(PO_3Me_2)-OH

The authors synthesized Boc-Abu(PO_3Me_2)-OH from commercially available Boc-Asp-O^{t}Bu in seven steps, and the synthesis is based on the formation of dimethyl

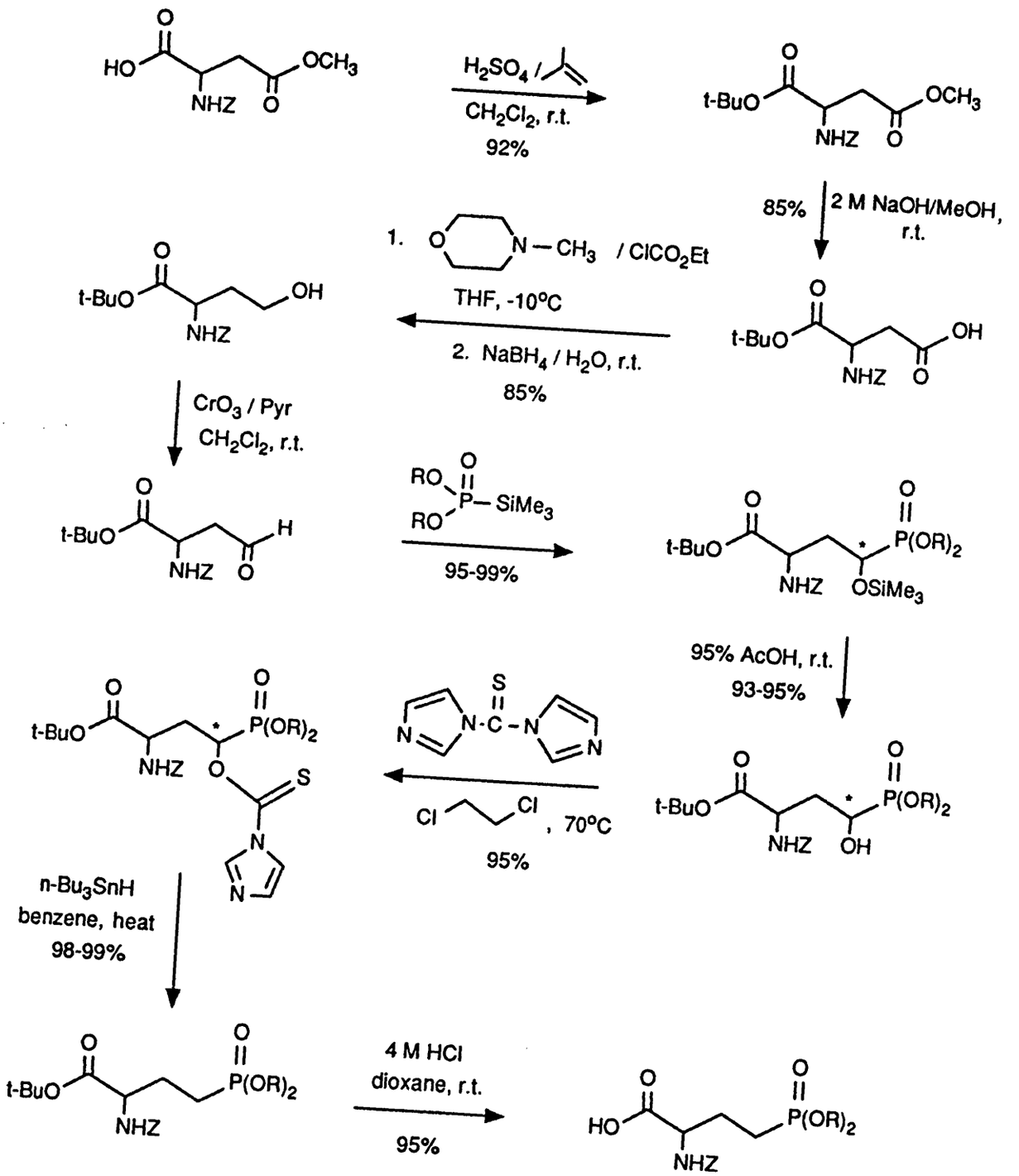
C-alkyl phosphonate group from the treatment of an aldehyde with dimethyl trimethylsilylphosphite (**Scheme 7**).

Valerio *et al.*[39] prepared stable peptide analogues of the highly-phosphorylated regions of human β-casein by replacing phosphoserine residues with the more stable 2-amino-4-phosphono butanoic acid residues. They devised a synthetic route for the preparation of the appropriate protected (S)-2-(*tert*-butyloxycarbonylamino) -4-dialkoxyphosphoryl butanoic acids from inexpensive (S)-aspartic acid. The key transformation in the synthesis involves reaction of a protected aspartaldehyde derivative with dialkyl trimethylsilyl phosphites to give the corresponding 2-amino-4-(dialkoxyphosphoryl)-4-trimethylsilyloxybutanoic acid derivatives (**Scheme 8**). Subsequently, they used these peptide analogues as haptens to elicit human β-casein specific monoclonal antibodies.

The 2-amino-4-phosphono butanoic acid, a naturally occurring glutamate, has also been reported to inhibit the binding of glutamate to "receptor like" hydrophobic proteolipids isolated from locust muscle, and to antagonise the excitatory action of glutamate applied iontophoretically to receptors present in the locust muscle membrane[40].


1. NMM / IBCF, THF, -20°C
2. NaBH4, CH3OH, 0°C
92%
NaOCl / TEMPO
2°C, 2h
79%
1. (CH3O)2P(O)Si(CH3)3/ CH3CN, 80°C, 1.5h
2. H2O, 80°C, 15 min
99%
1,1-Thiocarbonyl-diimidazole
1,2-dichoro-ethane, 50°C, 25 min
99%
Bu3SnH, benzene, 1.5h, 80°C
95%
CF3CO2H, 20°C, 1h
96%
(t-BuOCO)2O, Na2CO3, 0°C, 1h
66%
Boc-NH
O tBu
OH
H
(CH3O)2P
S
N
CF3CO2H.NH2

Scheme 7

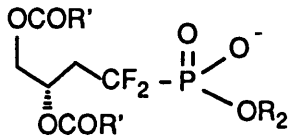

HO
NHZ
OCH3
H2SO4 /
CH2Cl2, r.t.
92%
t-BuO
85%
2 M NaOH/MeOH,
r.t.
1.
/ ClCO2Et
THF, -10°C
2. NaBH4 / H2O, r.t.
85%
OH
CrO3 / Pyr
CH2Cl2, r.t.
H
RO
SiMe3
95-99%
OSiMe3
P(OR)2
95% AcOH, r.t.
93-95%
S
70°C
95%
n-Bu3SnH
benzene, heat
98-99%
4 M HCl
dioxane, r.t.
95%

Scheme 8

1.7 Difluoro Functionalized Phosphonates

Organofluorine compounds have attracted the attention of investigators in various fields for their unique physiological and physical properties; for example in medicines, herbicides, and polymers.

Replacement of a phosphate functional group with a phosphonate moiety in biologically important molecules constitutes an attractive strategy for the design of non-hydrolyzable substrate analogues as inhibitors, or alternative substrates, for enzymes that process naturally-occuring phosphates[8]. Although the phosphonate moiety has frequently been employed as an isosteric replacement for the phosphate group, it has been proposed that the corresponding 1,1-difluoro alkyl phosphonate would be a superior replacement because this surrogate could more accurately mimic the steric and polar character of the phosphate function[8]. To evaluate this hypothesis, a number of studies have been conducted to examine the efficacy of 1,1-difluoroalkyl phosphonates as analogues of natural phosphates and, in many cases, these difluorophosphonates offered significant advantages over their nonfluorinated counterparts, as enzyme inhibitors or alternate substrates[8]. Martin *et al.*[8] prepared a series of 1,1-difluoroalkyl phosphonates related to compound (**29**) as potential inhibitors of phosphatidyl choline and phosphatidyl inositol specific phospholipase C's.



R' = alkyl or unsaturated alkyl
R_2 = choline or inositol

Compound (29)

Application of the C-C-P linkage has been of limited value. This limitation has been attributed, in part, to the failure of the α-CH_2 group in a phosphonate to adequately reflect the electronegativity of the oxygen and its influence on the adjacent phosphorus atom in a phosphate ester[16]. However, α-fluorination of alkyl-phosphonic acids, and their esters, heightens their similarity to the corresponding phosphonic acid esters. The use of fluorine as a biological tool is increasing, and analogues of biologically significant compounds can be prepared by replacing H, OH, Me, etc. with fluorine without introducing dramatic conformational changes[41]. Bergston *et al.*[42] investigated the synthesis and characterization of a new fluorine substituted non-ionic dinucleoside phosphonate analogue. They synthesized and characterized P-deoxy-P-(difluoromethyl) thymidylyl (3'→ 5') thymidine (**Figure 17**), as the first representative of a new class of phosphonate-linked nucleosides having a polar CF_2H group in place of the OH group of the natural phosphodiester linkage.

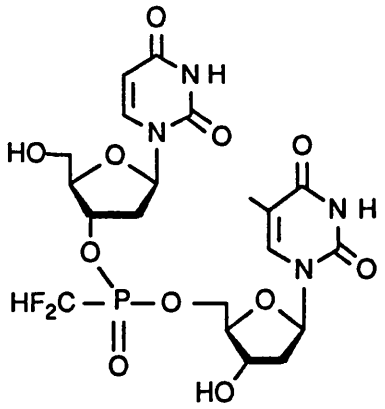


Figure 17 - P- deoxy -P- (difluoromethyl) thymidylyl (3'→5') thymidine

Fluorine is not a sterically demanding substituent, with its small Van der Waals radius (1.35 Å) close to that of hydrogen (1.20 Å)[43]. In molecules where conformational recognition is important, minimal steric disturbance by a substituent is

especially significant. Once introduced, the high carbon-fluorine bond energy renders the substituent relatively resistant to metabolic transformations. The eletronegativity of fluorine (4 vs 3.5 for oxygen) can have pronounced effects on the electron distribution in the molecule, affecting the basicity or acidity of neighboring groups, dipole moments within the molecule, and the overall reactivity and stability of neighboring functional groups. As a consequence of the available electron density, fluorine can function as a hydrogen bond acceptor. When this observation is considered, along with the fact that the carbon-fluorine bond length is 1.39 Å and the carbon-oxygen bond length 1.43 Å, it is clear that replacement of hydroxyl by fluorine in an analogue may be quite successful. Systematic substitution of fluorine can help establish the effect of hydroxylation or other metabolic processes on the action of the molecule, as has been successfully applied in the synthesis of fluorinated vitamin D_3 analogues[43].

In recent years, the difluoromethylene phosphonate group has attracted much attention due to its superior biological properties comparable to the analogous non-fluorinated phosphonates[44]. The analogues of pyro- and triphosphates, as well as glycerol-3-phosphate, in which the bridging oxygen atom was replaced by a difluoromethylene group, were successfully employed as substrates in enzymatic processes and as probes of proteins[41]. More recently, 9-(5,5-difluoro-5-phosphono pentyl) guanine (**Figure 18**) has been utilized as an analogue inhibitor of purine nucleoside phosphorylase[45].

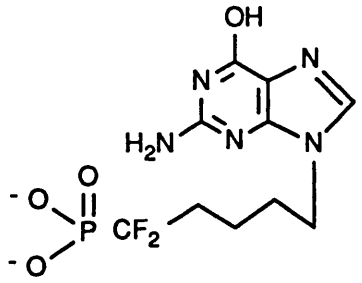
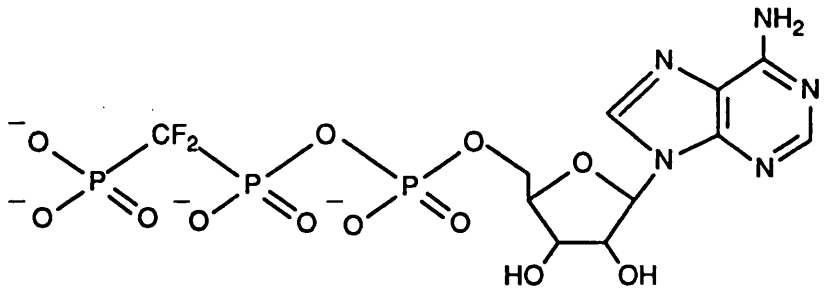
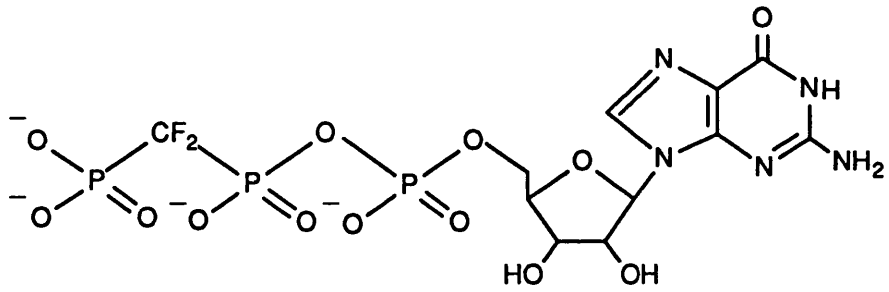


Figure 18 - 9-(5,5-difluoro-5-phosphono pentyl) guanine

Blackburn and co-workers[46] proposed that the difluoromethylene (CF_2) group may represent an isopolar, isosteric replacement for oxygen in the polyphosphate moiety of nucleotides. For example, among a series of β,γ-substituted ATP and GTP derivatives (**Figure 19**), those with the CF_2 group replacing the β,γ-phosphoanhydride oxygen were most similar in chemical and physical properties to the natural nucleotides.



analogue of adenosine 5'-triphosphate (ATP)

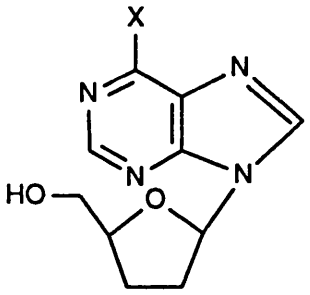


analogue of GTP

Figure 19 - Analogues of ATP and GTP

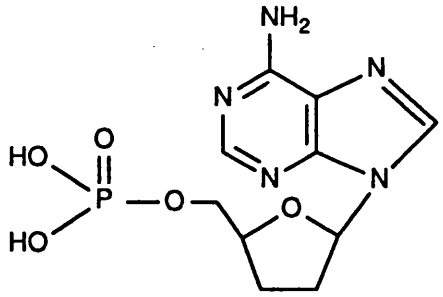
2',3'-Dideoxyinosine (ddI) (**31**) and 2',3'-dideoxyadenosine (ddA) (**30**) are considered to be the most effective agents to prevent HIV replication. Both of these compounds are believed to exert their antiviral effect because they are intracellularly transformed into 2',3'-dideoxyadenosine triphosphate (ddATP) which is a potent

inhibitor of HIV reverse transcriptase. 2',3'-Dideoxyadenosine monophosphate (ddAMP)(32) has been identified as a common key intermediate in the metabolic process required for antiviral activity[47]. These consideration led Wolff-Kugel *et al.*[47] to design new stable synthetic analogues of ddAMP (32) as potential anti-HIV agents, by replacing the oxygen in the sugar ring by a methylene group, (in order to avoid the metabolic instability due to the glycosidic bond), and by replacing the labile phosphate monoester of ddAMP by a phosphonate group (33) (Y= CH_2O, CF_2, CH_2).

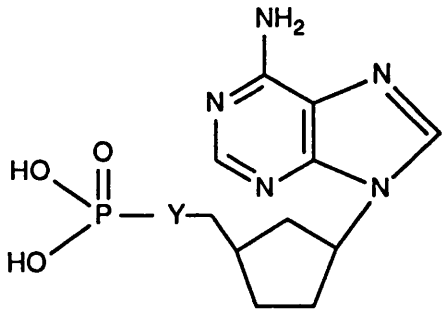


(30) ddA (X= NH_2)

(31) ddI (X= OH)



(32) ddAMP

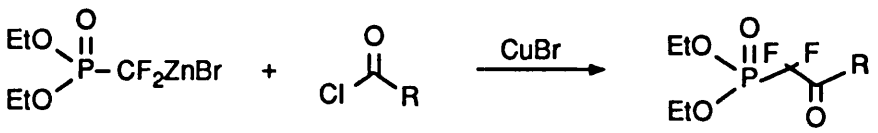


(33) Y = CH_2O, CF_2, CH_2

The preparation and chemistry of organofluorine compounds have received considerable attention. However, there are a lack of methods for the preparation of difluorofunctionalized phosphonates.

Burton *et al.*[7,48] reported the preparation of ethyldifluoro(diethoxy phosphinyl) acetate (34) by the acylation of [(diethoxyphosphinyl)difluoromethyl] zinc bromide with ethyl chloroformate, in the presence of a catalytic amount of cuprous

bromide. Ethyl difluoro (diethoxyphosphinyl) pyruvate (35), and N,N-diethyldifluoro (diethoxyphosphinyl) acetamide (36), were similarly prepared (**Scheme 9**).

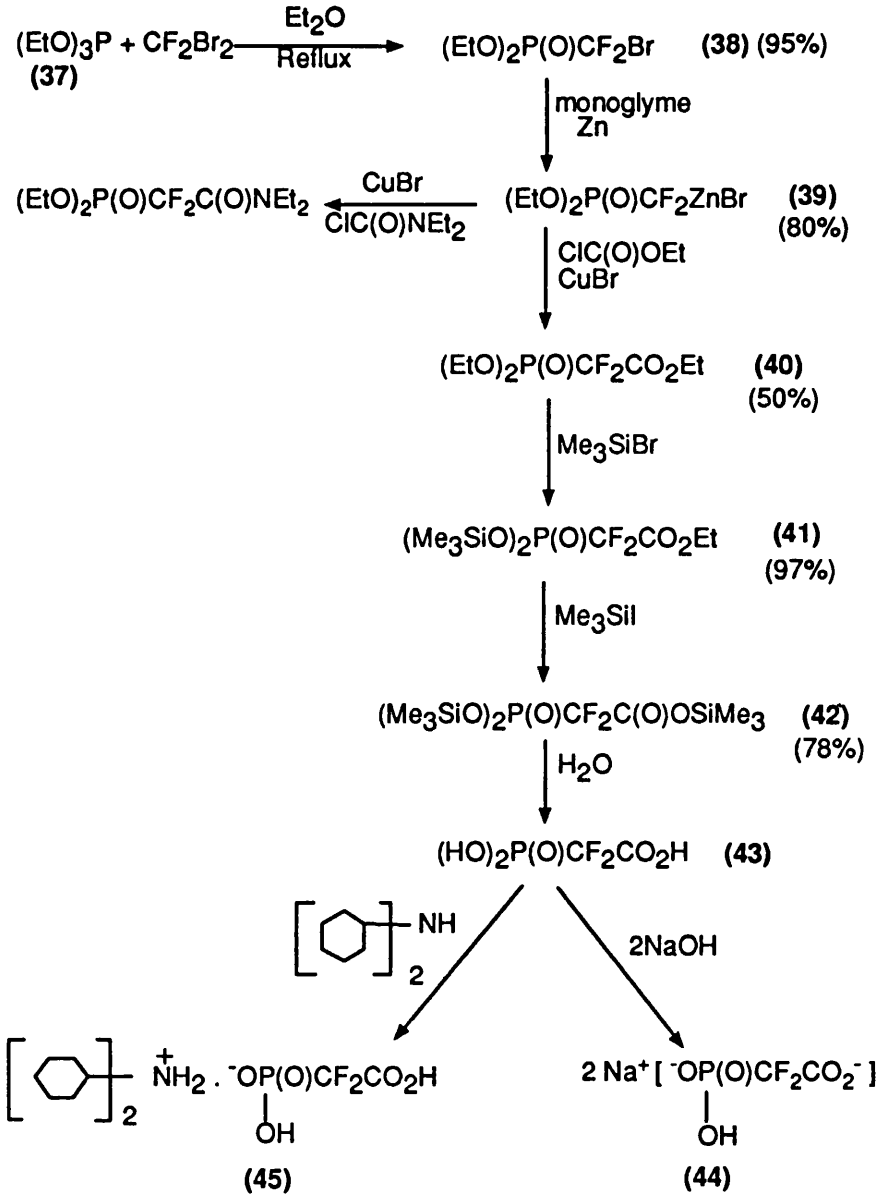


	Yield
(34) R = OEt	50%
(35) R = C(O)OEt	62%
(36) R = NEt_2	38%

Scheme 9

Burton *et al.*[49] also reported a safe and facile synthesis of difluoro phosphoacetic acid (**Scheme 10**). First, diethyl (bromo difluoromethyl)phosphonate (**38**) was prepared from triethylphosphite (**37**) and dibromodifluoromethane. Reaction of (**38**) with zinc dust gave the stable [(diethoxyphosphinyl) difluoromethyl] zinc bromide (**39**) which will react with acyl chlorides to yield (2-oxo-1,1-difluoroalkyl)phosphonates. However, acylation of (**39**) with ethyl chloroformate gave little or no product (**40**), whereas catalysis with cuprous bromide gave a smooth reaction of (**39**) and ethyl chloroformate to provide a good yield of (**40**). Similar catalysis permitted the acylation of (**39**) with diethylcarbamoyl chloride to give the corresponding amide derivative.

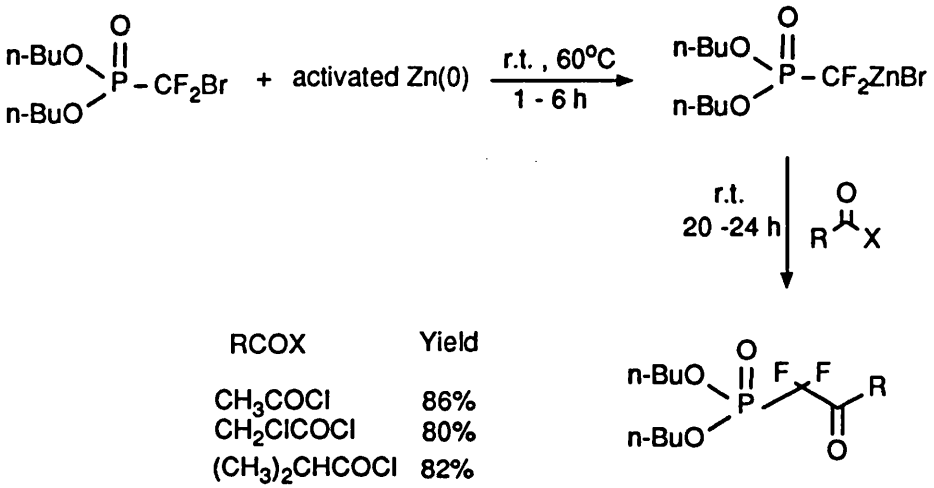
Conversion of (**40**) to (**43**) was accomplished via selective silylation of (**40**) at the phosphonic ester to give (**41**). Further silylation of (**41**) with the more reactive iodotrimethylsilane gave the trisilylated ester (**42**). Dissolution of (**42**) in water immediately gave (**43**) in quantitative yield; fluoroacid (**43**) is extremely hygroscopic but was isolated as a white crystalline monoamine salt (**45**), or as a stable monohydrate of the disodium salt (**44**) of (**43**).



Scheme 10

Burton *et al.*[50] also described the reaction of diethyl bromodifluoromethylphosphonate with cadmium to form a stable cadmium complex. Depending on solvent, this functionalized organocadmium reagent exhibits stability for days to months. It reacts with a variety of electrophiles and serves as a synthetical useful source for the introduction of the difluoromethylene phosphonate group into organic compounds.

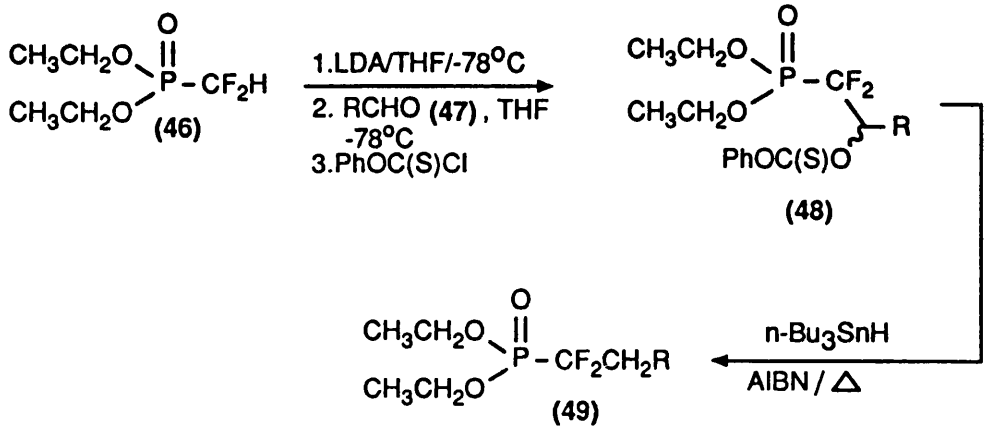
Burton *et al.*[51] reported the treatment of dialkyl bromodifluoromethyl phosphonate with activated zinc dust in various solvents to give dialkoxy phosphinyl difluoromethyl zinc compounds which were acylated with acid halides, or fluorinated acid anhydrides, to afford 2-oxo-1,1-difluoroalkyl phosphonates in good yields (**Scheme 11**).



RCOX	Yield
CH_3COCl	86%
$CH_2ClCOCl$	80%
$(CH_3)_2CHCOCl$	82%
PhCOBr	62%
$(CF_3CO)_2O$	77%

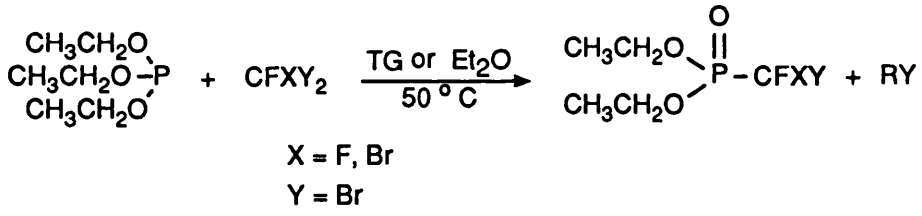
Scheme 11

Martin *et al.*[8] developed a facile method for the preparation of 1,1-difluoroalkylphosphonates (**49**). Metallation of (**46**) with lithium diisopropylamine at -78°C, followed by reaction with various aldehydes (**47**), afforded an alkoxide intermediate that was trapped *in situ* with phenyl chlorothionoformate to give the thionocarbonates (**48**) in 71-90% yields. Subsequent Barton deoxygenation of (**48**) proceeded smoothly upon treatment with tri-n-butyltin hydride, in the presence of AIBN in refluxing toluene, to provide the desired 1,1-difluoroalkylphosphonates (**49**) in 81-89% yields (**Scheme 12**).



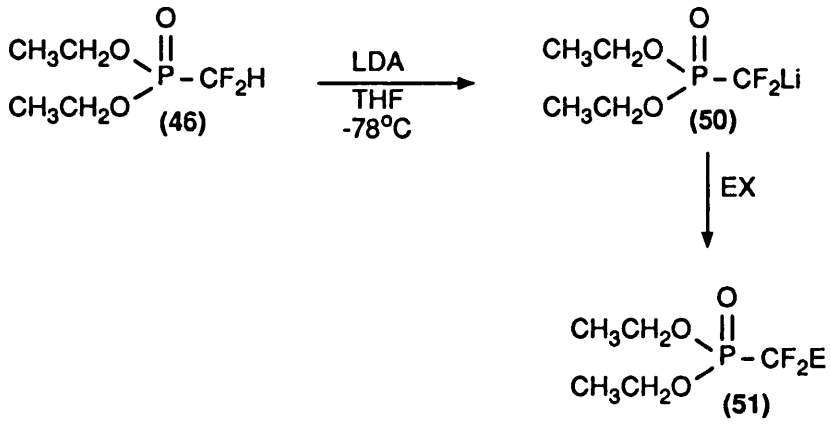
Scheme 12

Burton *et al.*[52] reported that dibromofluoromethane undergoes a facile Michaelis-Arbuzov reaction with alkyl phosphites to give the corresponding bromo-fluoro-methylphosphonate esters. The reactions are conveniently carried out in triglyme (TG) or diethyl ether (Et_2O) at 25°C to 50°C, and give good yields of the phosphonate esters (**Scheme 13**).



Scheme 13

Obayashi *et al.*[53] prepared (diethylphosphinyl) difluoromethyl lithium by treatment of diethyl difluoromethylphosphonate (36) with lithium diisopropylamine (LDA) in THF at -78°C. (Diethylphosphinyl) difluoromethyl lithium reacts with various electrophiles to yield compounds having difluoromethylene or difluoromethyl groups (**Scheme 14**).



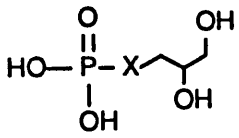
Where :

E= Me_3Si, n-Bu_3Sn, Et, C_6H_5,CH_2=CH-CH_2 , C_6H_5CO

X= Cl, Br

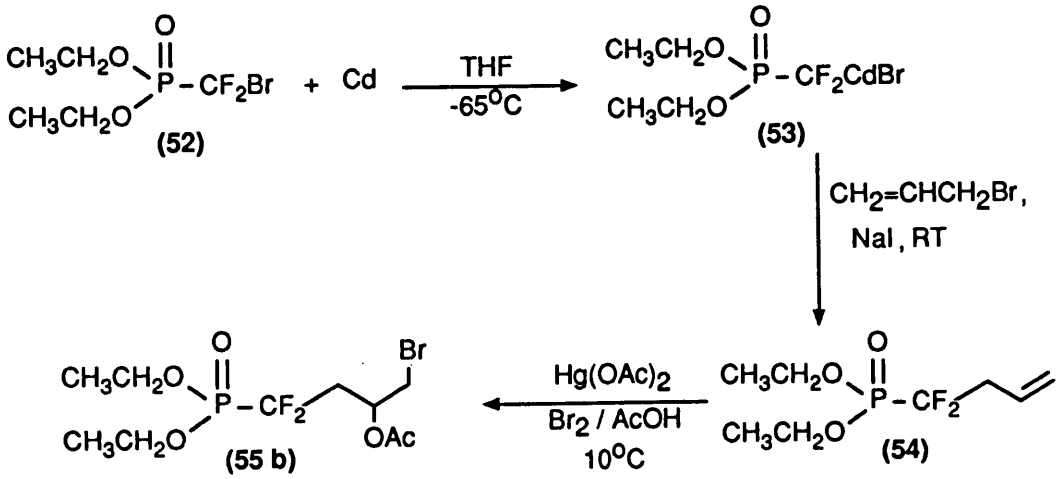
Scheme 14

Chambers *et al.*[41] synthesized a number of difluoromethylene phosphonates as phosphate analogues, and tested them in appropriate biological systems. One was 1,1-difluoro-3,4-dihydroxybutyl phosphonic acid (**58**), a structural analogue of glycerol-3-phosphate (**59**), prepared using a modification of the procedure described by Burton and his co-workers[50]. Reaction of cadmium with bromo difluoromethyl phosphonate (**52**) generates the organocadmium reagent (**53**). Reaction of (**53**) with allyl bromide gave the derivative (**54**) which, with bromine and mercuric acetate, provided a 50:50 mixture of bromo-acetates (**55a**) and (**55b**). The oxirane (**56**) was obtained in moderate yield when this mixture was stirred in methanolic potassium hydroxide. Acid-catalysed ring opening afforded diol (**57**) which was treated with bromotrimethylsilane for conversion into the corresponding bistrimethylsilyl ester, and aqueous hydrolysis then provided the free acid (**Scheme 15**).



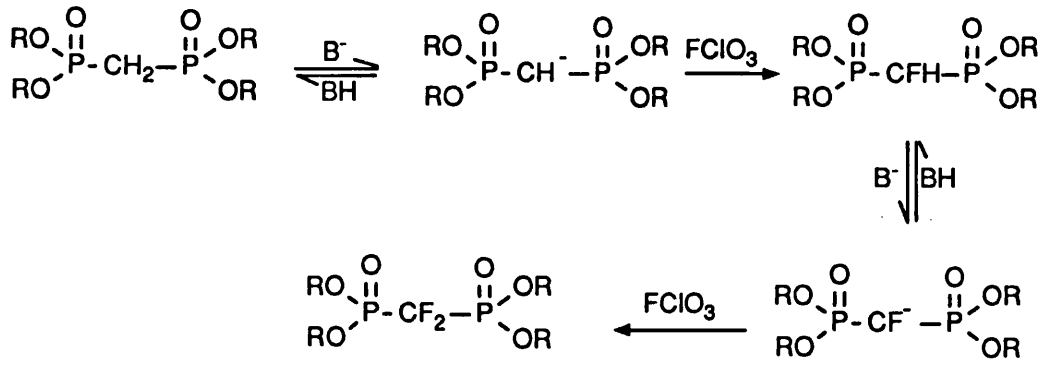
(58) X = CF_2

(59) X = O



Scheme 15

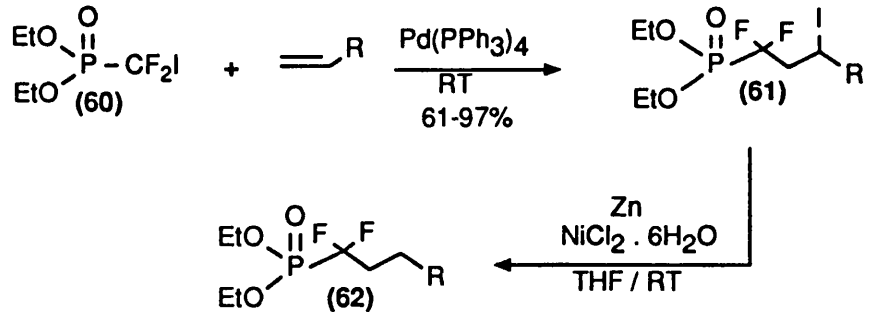
McKenna *et al.*[54] found that perchloryl fluoride reacts smoothly with tetraisopropyl or tetraethyl methanediphosphonate carbanion in dry toluene, to form both the correponding fluorophosphonate and difluorophosphonate esters in total yields of up to 85% when potassium tert-butoxide is used as the base instead of NaOEt (**Scheme 16**).



Scheme 16

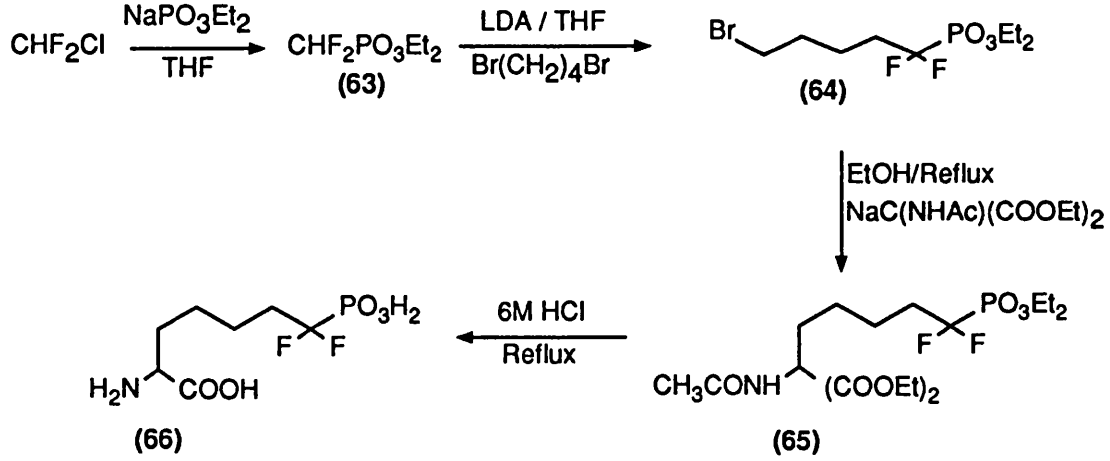
The fluorination reaction proceeds virtually as a titration of base with perchloryl fluoride and shows a readily recognizable end point marked by a characteristic colour change from dark to pale yellow. Termination of the reaction is also indicated by the end of the temperature rise that accompanies the reaction, and the cessation of perchloryl fluoride uptake.

Burton *et al.*[44] have developed a novel and general method to prepare α,α-difluoro functionalized phosphonates (**62**): the diethyl iododifluoromethyl phosphonate (**60**) reacts with alkenes in the presence of catalytic amounts of tetrakis (triphenyl phosphine) palladium in the absence of solvent, to afford excellent yields of the corresponding adducts (**61**) at room temperature within 30 minutes. Then the adduct (**61**) can be readily converted to the α,α- difluorophosphonate by treatment with zinc in the presence of nickel chloride hexahydrate in moist THF at room temperature (**Scheme 17**).



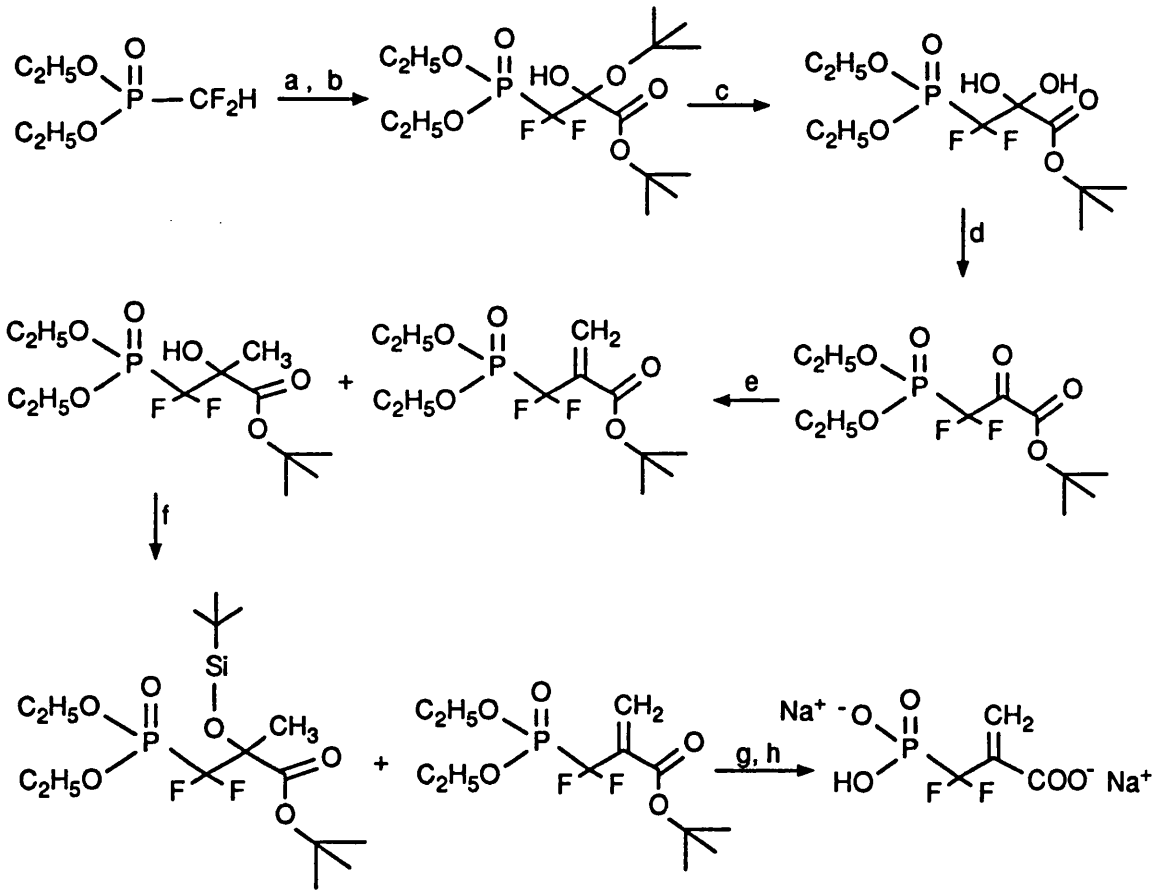
Scheme 17

Bigge *et al.*[55] reported the synthesis of 2- amino- 7,7- difluoro -7-phosphonoheptanoic acid (**66**). Chlorodifluoromethane was treated with sodium diethyl phosphonate (**63**) to provide (**66**) as a volatile oil in good yield. Deprotonation with lithium diisopropylamine, followed by addition of excess 1,4-dibromobutane at -78°C, gave a moderate yield of the desired monoalkylated product (**64**). Displacement of the remaining bromine with sodium acetamidomalonate proceeded smoothly to give (**65**). Refluxing this product with 6M hydrochloric acid resulted in the removal of the protecting groups and malonate decarboxylation to afford (**66**) (**Scheme 18**).



Scheme 18

Phillion *et al.*[56] reported the synthesis of the difluoromethylene analogue of phosphoenolpyruvate. They synthesized the disodium salt of 2-[(dihydroxyphosphinyl) difluoromethyl] propenoic acid (**Scheme 19**).



Reactions conditions:

(a) LDA, <-70°C; (b) di-tert-butyl oxalate, <-70°C, then acidic workup; (c) saturated aqueous $NaHCO_3/CH_3CN$; (d) benzene azeotrope; (e) Tebbe reagent; (f) N,O-Bis-(trimethylsilyl)-trifluoroacetamide, rt for 6d; (g) TFA, 8h at reflux; (h) $NaHCO_3$.

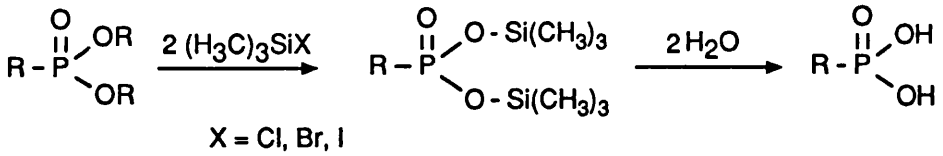
Scheme 19

1.8 Hydrolysis of Difluorophosphonate Compounds

The hydrolysis of organophosphorus compounds usually results in derivatives with specific properties for practical application, e.g. for use in a strong alkaline dip, in acid soils, and to regulate the rate of degradation in plants or animals (residue tolerances, waiting periods, toxicological effects, etc.)[57].

Difluorophosphonates can be hydrolyzed to give the corresponding acids by efficient methods that include the reaction with iodotrimethylsilane (chloro- or bromotrimethylsilane), followed by aqueous hydrolysis.

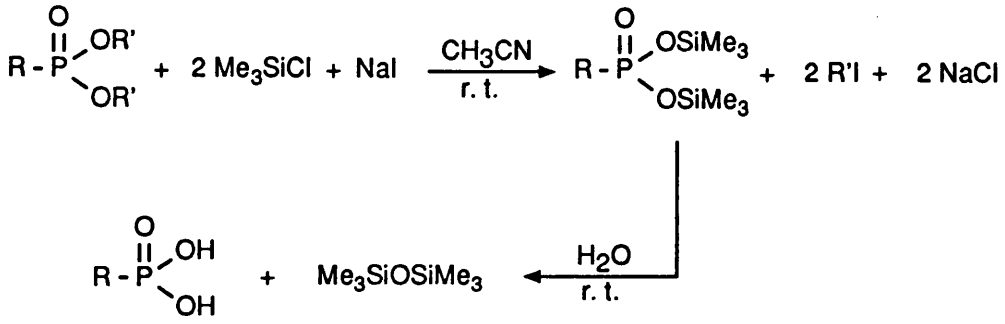
The reaction is believed to occur via a mechanism involving nucleophilic attack on silicon by the phosphonyl oxygen, followed by dealkylation of a phosphonium intermediate[58]. The products formed in this way are of interest because oxygen-bridged silyl groups attached to phosphorus are readily removable under extremely mild conditions, leading to the formation of the free phosphonic acids[59](**Scheme 20**).



Scheme 20

Blackburn *et al.*[60] showed that bromo- and chloro-trialkylsilanes interact with trialkylphosphates, dialkylphosphonates and alkyl carboxylic esters to give the corresponding trialkylsilyl esters and trialkylsilyl carboxylates which, in turn, can be hydrolysed readily to afford the parent phosphorus acids. Later McKenna *et al.*[61] established that the use of bromotrimethylsilane is superior to that of chlorotrimethylsilane. Furthermore, it appeared likely that iodotrimethylsilane would exhibit even greater reactivity towards phosphate and phosphonate alkyl esters[62].

Morita *et al.*[62] and Olah *et al.*[63] developed a convenient method for dealkylation of dialkylphosphonates by chlorotrimethylsilane in the presence of sodium iodide. The bromotrimethylsilane was added to phosphonates in the presence of sodium iodide in acetonitrile, with stirring at room temperature for 15 minutes, then an exothermic reaction occurred immediately resulting in the precipitation of sodium chloride to afford the corresponding silyl phosphonates in good yields (**Scheme 21**).



When R = MeCO and R' = Et, some further warming up to 40°C, for 15 minutes, was required

Scheme 21

Blackburn *et al.*[60] reported that iodotrimethylsilane is unquestionably superior in use to the combination of chlorotrimethylsilane and sodium iodide as described by Morita *et al.*[62], both with respect to the ease of the operations involved, and with regard to its greater specificity. McKenna *et al.*[61] reported that the chlorotrimethylsilane-sodium iodide treatment of diethylbromomethyl phosphonate leads to a mixture of halogenomethyl phosphonates, but the reaction with iodotrimethylsilane gives only trace amounts of iodinated phosphonate in the crude reaction product, which are readily removed by crystallization.

CHAPTER TWO

RESULTS AND DISCUSSION

CHAPTER TWO

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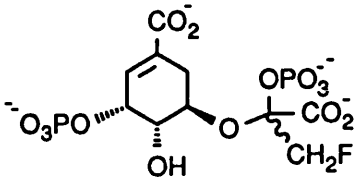
2.1 Aims and Objectives

The objective of this project was to synthesise new organophosphorus compounds in which the C-O-P group is replaced by C-CF_2-P moiety, in order to explore the potential of fluorophosphonates as phosphate analogues, and to test them in appropriate biological systems.

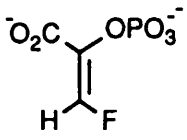
Earlier studies on difluorophosphonates have been described in Chapter One, and we would refer in particular to the work of Blackburn[64] who described difluorophosphonates as isosteric and isopolar mimics of biologically important phosphates. Blackburn showed that the fluorophosphonate unit has steric and electronic properties much closer to the natural phosphate than the corresponding non-fluorinated ones, which contain CH_2P as a surrogate for OP.

Phosphoenolpyruvate (PEP) plays an important role in the shikimic acid pathway for the formation of 5-enolpyruvylshikimate-3-phosphate (5-EPS-3-P) (**11**), which is enzymatically synthesized by the nucleophilic attack of the 5-OH of the shikimate 3-phosphate (S3P) on the C-2 position of PEP with the elimination of phosphate[65] (**Figure 20**). The reaction proceeds through a tetrahedral intermediate (**13**) which has been previously isolated and characterized by Anderson[12]. Structural mimics of this intermediate are indeed potent EPSPS inhibitors[10].

evidence that (Z)-3-fluoro-PEP (**120**) functions as pseudosubstrate for 5-EPS-3-P synthase producing, in one step the unexpected monofluoro analogue (**121**), which remains tightly bound at the enzyme active site



(**121**)



(**120**)

The replacement of the C-O-P group in the phosphoenolpyruvate by C-CF_2-P is one strategy used to stabilise the ketal phosphate structure of the tetrahedral intermediate (**13**), and gives some stable analogues that could be potential inhibitors of EPSP synthase.

An objective of this project was to synthesize a new class of difluorovinyl phosphonate analogues of PEP: methyl 2-[2',2'-difluoroethyl-2'-(diethoxyphosphinyl)] propenoate (**67**), 2-[2',2'-difluoroethyl-2'-(diethoxyphosphinyl)] propenoic acid (**68**), E-[4,4-difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid (**71**), Z-[4,4-difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid (**72**), and 2-[2',2' -difluoroethyl-2'- (dihydroxy phosphinyl)] propenoic acid (**69**)). It is hoped that this class of compounds will provide important inhibitors of the shikimic acid pathway. Certain of these could be envisaged as potential Michael acceptors which could bind irreversibly to an enzyme active site for which PEP is a substrate[56].

Our proposed routes to the target compounds are outlined in **Scheme 22.**

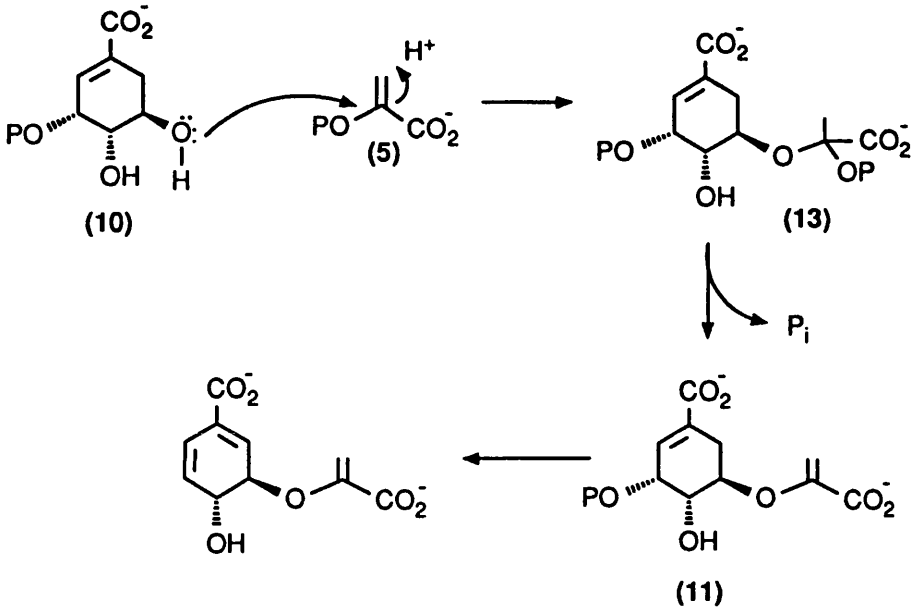
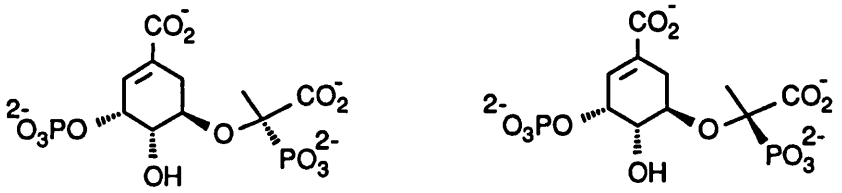


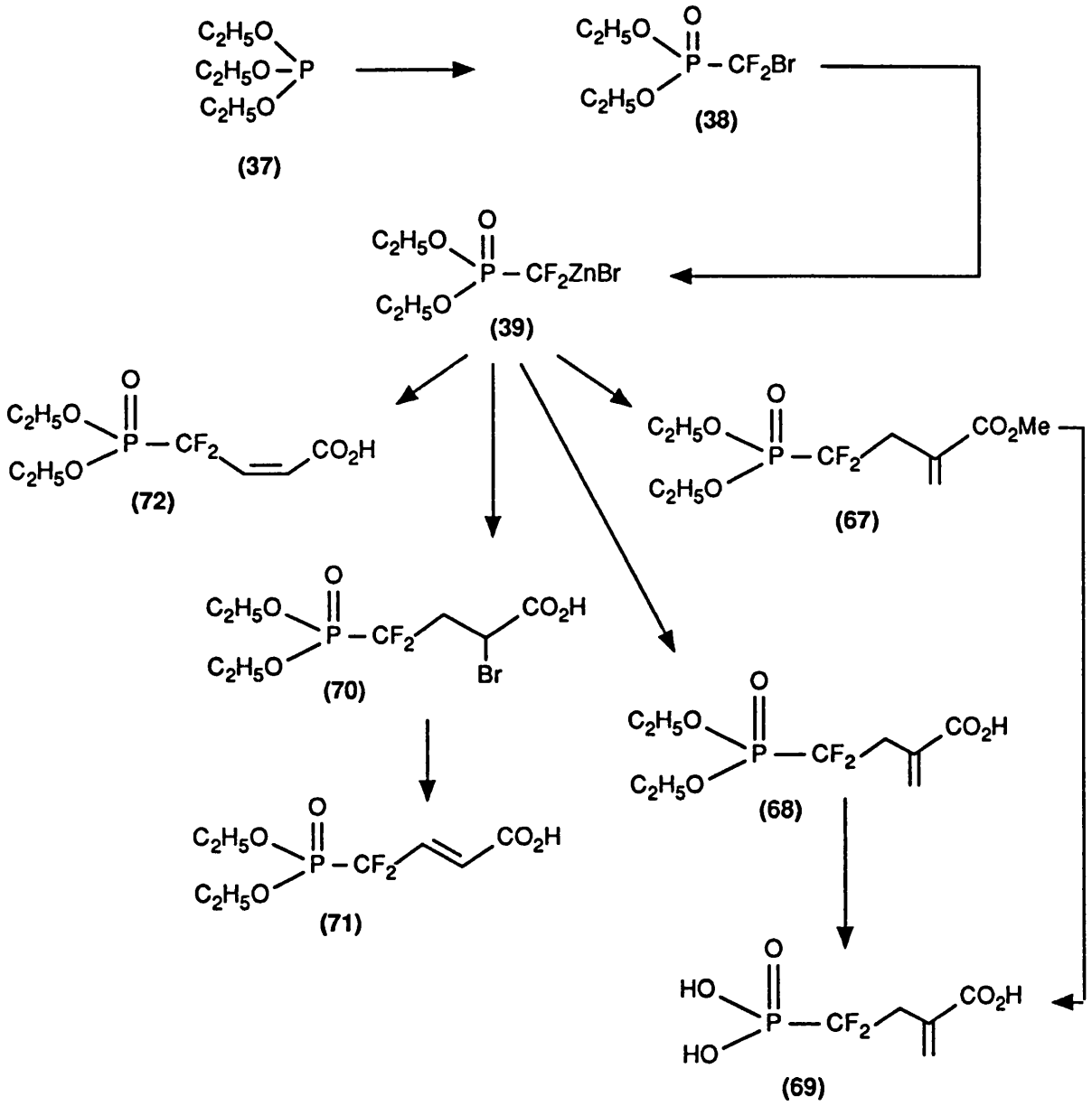
Figure 20 - Proposed Mechanism of 5- EPS -3- P Synthase

The tetrahedral intermediate (**13**), although stable under alkaline conditions, hydrolyses readily at neutral pH, and the configuration of the ketal carbon has not been elucidated[65]. It also decomposes under acidic conditions to form pyruvate and S3P[12].

Bartlett *et al.*[11] reported the synthesis of two diastereoisomers of phosphonate (**118** and **119**), a stable analogue of the tetrahedral intermediate, and their evaluation as inhibitors of EPSP synthase.



There are a variety of PEP analogues that have been examined as alternate substrates and/or inhibitors of 5-EPS-3-P synthase. Walker *et al.*[66] reported the first



Scheme 22

Phosphoenolpyruvate and some of its analogues are also important inhibitors of prolidase. Prolidase is a manganese-dependent hydrolase that cleaves dipeptides, in which the amide bond of the nitrogen atom of proline is in the trans-configuration. This enzyme is present in microorganisms and many mammalian tissues, where it is believed to catalyze terminal degradation of exogenous and endogenous proteins, permitting recycling or renal excretion of proline and hydroxyproline. In humans, a deficiency of prolidase results in a complex clinical syndrome involving mental retardation[67].

Radzicka *et al.*[67] examined a variety of carboxylic, phosphoric, and phosphonic acids in designing new inhibitors of prolidase (**Table I**).

According to these authors, both compounds related to 1,2-cyclopentanedicarboxylic (**Figure 21**), and 1,2 cyclohexanedicarboxylic acids serve as effective inhibitors of prolidase.

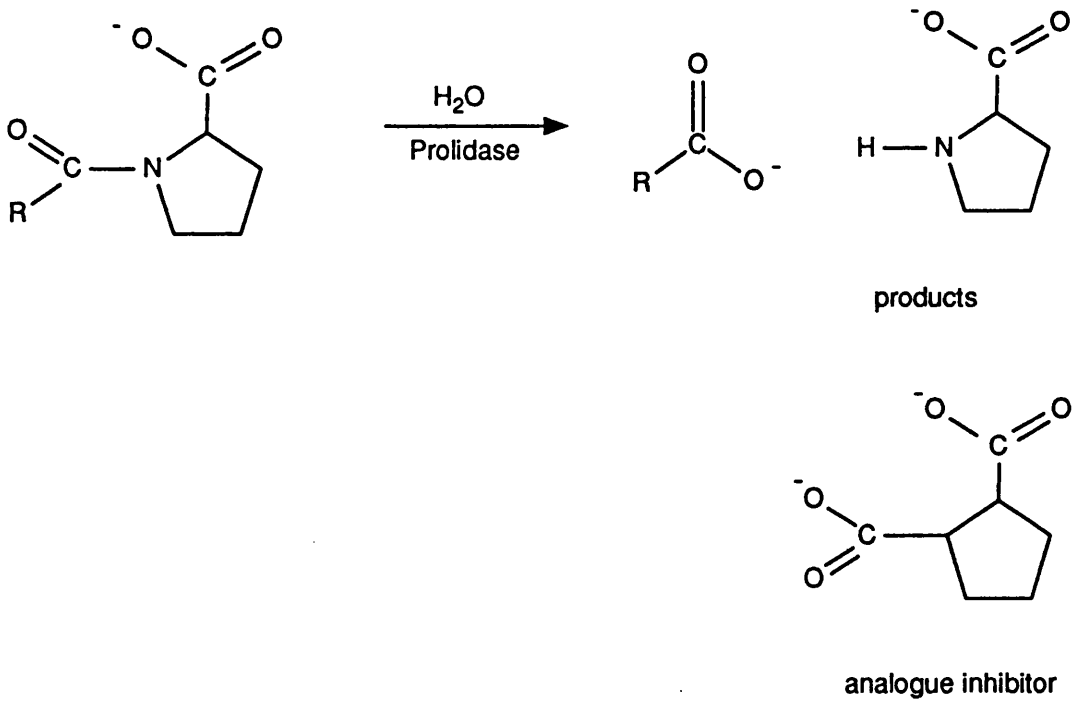


Figure 21 - Inhibitors of Prolidase

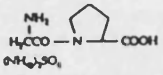
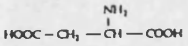
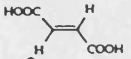
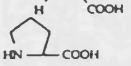
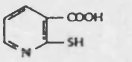
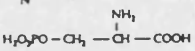
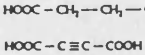
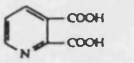
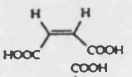
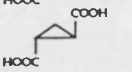
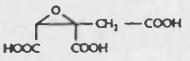
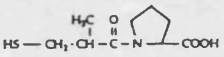
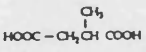
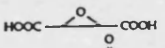
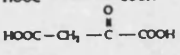
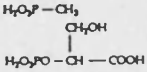
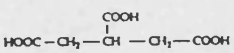
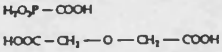
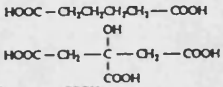
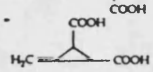
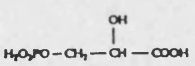
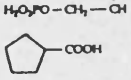
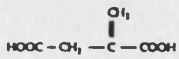
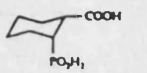
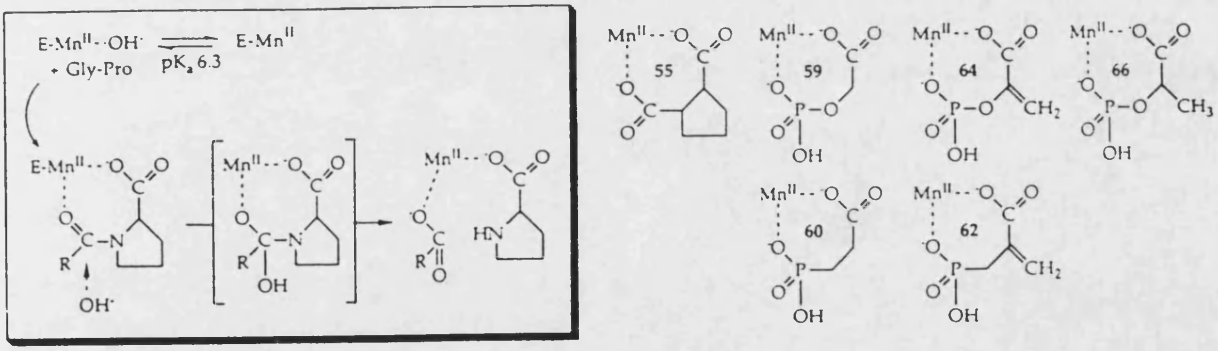
#	Name	Structure	K_i [M]
1.	Acetic acid		1.1×10^{-2}
2.	Gly-Pro		1.2×10^{-3}
3.	Ammonium sulphate		9.6×10^{-3}
4.	1-Aminocyclopentane--1-carboxylic acid		$>5\times10^{-3}$
5.	DL-Aspartic acid		1.3×10^{-3}
6.	Fumaric acid		$>1\times10^{-3}$
7.	L-Proline		7.0×10^{-4}
8.	3-Mercaptopropionic acid		6.3×10^{-4}
9.	2-Mercaptonicotinic acid		5.6×10^{-4}
10.	DL-Serine phosphate		5.1×10^{-4}
11.	Succinic acid		4.9×10^{-4}
12.	Acetylenedicarboxylic acid		4.5×10^{-4}
13.	2,3-Pyridinedicarboxylic acid		4.4×10^{-4}
14.	Maleic acid		4.1×10^{-4}
15.	DL-*trans*-1,2-Cyclopropanedicarboxylic acid		4.0×10^{-4}
16.	Malonic acid		4.0×10^{-4}
17.	(±)-*cis*-Epoxytricarballylic acid		3.5×10^{-4}
18.	Hexafluoroglutaric acid		3.1×10^{-4}
19.	Captopril		2.7×10^{-4}
20.	Methylsuccinic acid		2.2×10^{-4}
21.	Oxalic acid		2.1×10^{-4}
22.	Isophthalic acid		2.0×10^{-4}
23.	Ethylenediphosphonic acid		1.7×10^{-4}
24.	2,3-Epoxysuccinic acid		1.6×10^{-4}
25.	Oxalacetic acid		1.6×10^{-4}
26.	Methylphosphonic acid		1.6×10^{-4}
27.	D(+)-2-Phosphoglyceric acid		1.3×10^{-4}
28.	Tricarballylic acid		1.3×10^{-4}
29.	Phosphonoformic acid		1.2×10^{-4}
30.	Diglycolic acid		1.2×10^{-4}
31.	Adipic acid		8.3×10^{-5}
32.	Citric acid		7.4×10^{-5}
33.	3-Methylenecyclopropane-*trans*-1,2-dicarboxylic acid		7.2×10^{-5}
34.	DL-3-Phosphoglyceric acid		7.1×10^{-5}
35.	Cyclopentanecarboxylic acid		6.2×10^{-5}
36.	Glutaric acid		6.1×10^{-5}
37.	Itaconic acid		4.7×10^{-5}
38.	3,4-Furandicarboxylic acid		3.8×10^{-5}
39.	2-Phosphinocyclohexanecarboxylic acid		2.4×10^{-5}
40.	N-(Phosphonomethyl)-glycine		2.3×10^{-5}
41.	Methylenediphosphonic acid		1.8×10^{-5}
42.	Thiodiglycolic acid		1.5×10^{-5}
43.	(±)*trans*-1,2-Cyclobutanedicarboxylic acid		1.5×10^{-5}
44.	Propylenediphosphonic acid		1.3×10^{-5}
45.	2-Ketoglutaric acid		1.2×10^{-5}
46.	3-Hydroxy-3-methylglutaric acid		1.1×10^{-5}
47.	*cis*-1,2-Cyclobutanedicarboxylic acid		8.5×10^{-6}
48.	4-Phosphonobutyric acid		2.4×10^{-6}
49.	2-Carboxyphenyl phosphate		1.7×10^{-6}
50.	Phthalic acid		1.2×10^{-6}
51.	(Z)-3-Fluoromethylphosphoenolpyruvic acid		9.7×10^{-7}
52.	*cis*-1,2-Cyclohexanedicarboxylic acid		7.9×10^{-7}
53.	Phosphonoacetic acid		5.8×10^{-7}
54.	Homophthalic acid		5.3×10^{-7}
55.	*trans*-DL-1,2-Cyclopentanedicarboxylic acid		5.1×10^{-7}
56.	(*E*)-3-Cyanophosphoenolpyruvic acid		2.0×10^{-7}
57.	*trans*-DL-1,2-Cyclohexanedicarboxylic acid		1.3×10^{-7}
58.	2-Phosphonocyclohexanecarboxylic acid		9.25×10^{-8}
59.	Phosphoglycolic acid		8.8×10^{-8}
60.	3-Phosphonopropionic acid		7.2×10^{-8}
61.	(Z)-3-Fluorophosphoenolpyruvic acid		4.5×10^{-8}
62.	2-(Phosphonomethyl)-acrylic acid		1.3×10^{-8}
63.	(Z)-3-Chlorophosphoenolpyruvic acid		9.6×10^{-9}
64.	Phosphoenolpyruvic acid		8.5×10^{-9}
65.	(Z)-3-Methylphosphoenolpyruvic acid		6.2×10^{-9}
66.	Phospholactic acid		5.0×10^{-9}
67.	(Z)-3-Bromophosphoenolpyruvic acid		4.6×10^{-9}

Table I Inhibitors of Pig Kidney Prolidase

Phospho- and phosphonocarboxylic acids proved to be more potent inhibitors than the corresponding dicarboxylic acids, but diphosphonic acids although, more inhibitory than dicarboxylic acids, were less effective than phospho- and phosphonocarboxylic acids. The exceptional potencies conferred on carboxylic acids by the presence of a single phospho and phosphono group may be due to their higher affinities for an active-site metal ion, and/or to the possibility that the single phospho or phosphono group has a tetrahedral configuration at the phosphorus that resembles an sp^3-hybridized intermediate in peptide hydrolysis.

The derivatives of phosphoenolpyruvic acid, fluorinated, chlorinated or brominated, are among the most effective inhibitors of prolidase, and 2-(phosphono methyl) acrylic acid (nomenclature according to the author)(**62** in **Table I**), is one of them. One of our targets, 2-[2',2'-difluoroethyl-2'-(dihydroxyphosphinyl)] propenoic acid (**69**), which is an analogue of 2-(phosphonomethyl) acrylic acid, could act as a potential inhitor of prolidase.

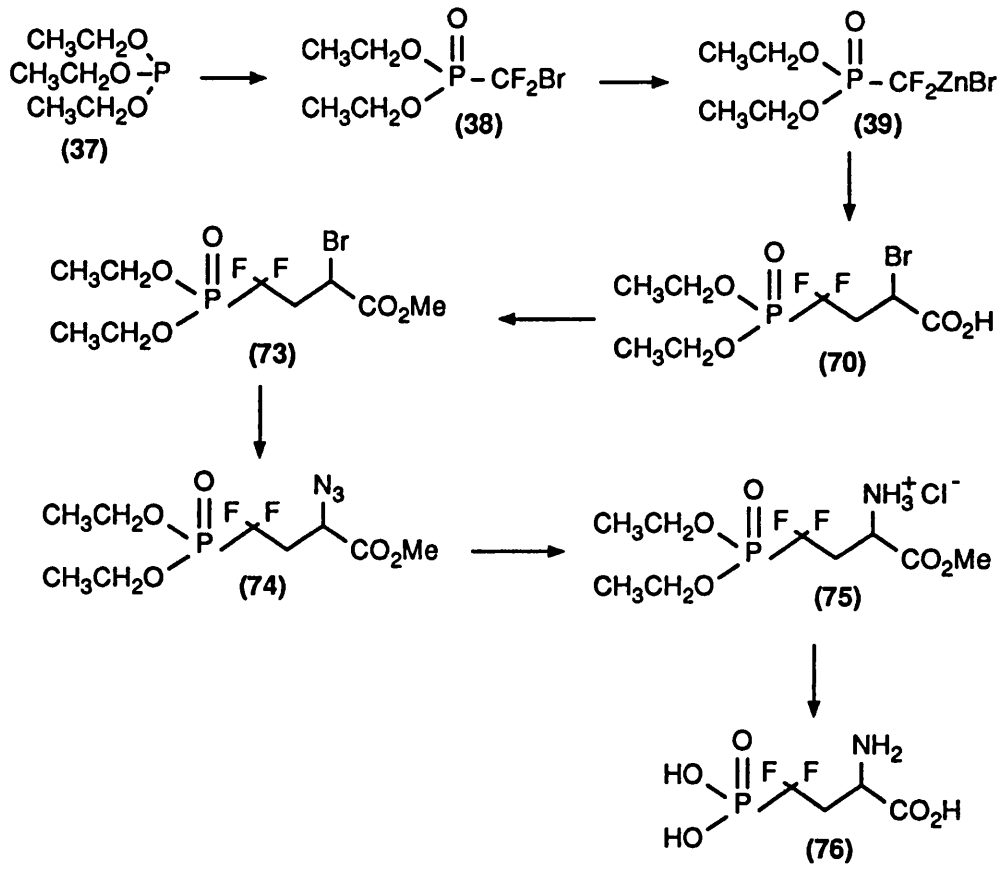
Scheme 23 shows some possible binding interactions of substrates, products and inhibitors with manganese at the active site of prolidase, as proposed by Radzicka et al[67].



One Potential Mechanism of Action of Prolidase, Showing the Resemblence of the Possible Intermediate Shown in Brackets to Enzyme Complexes Formed by Anions of Several Inhibitors That May Displace Hydroxide Ion from Manganese at the Enzymes's Active Site

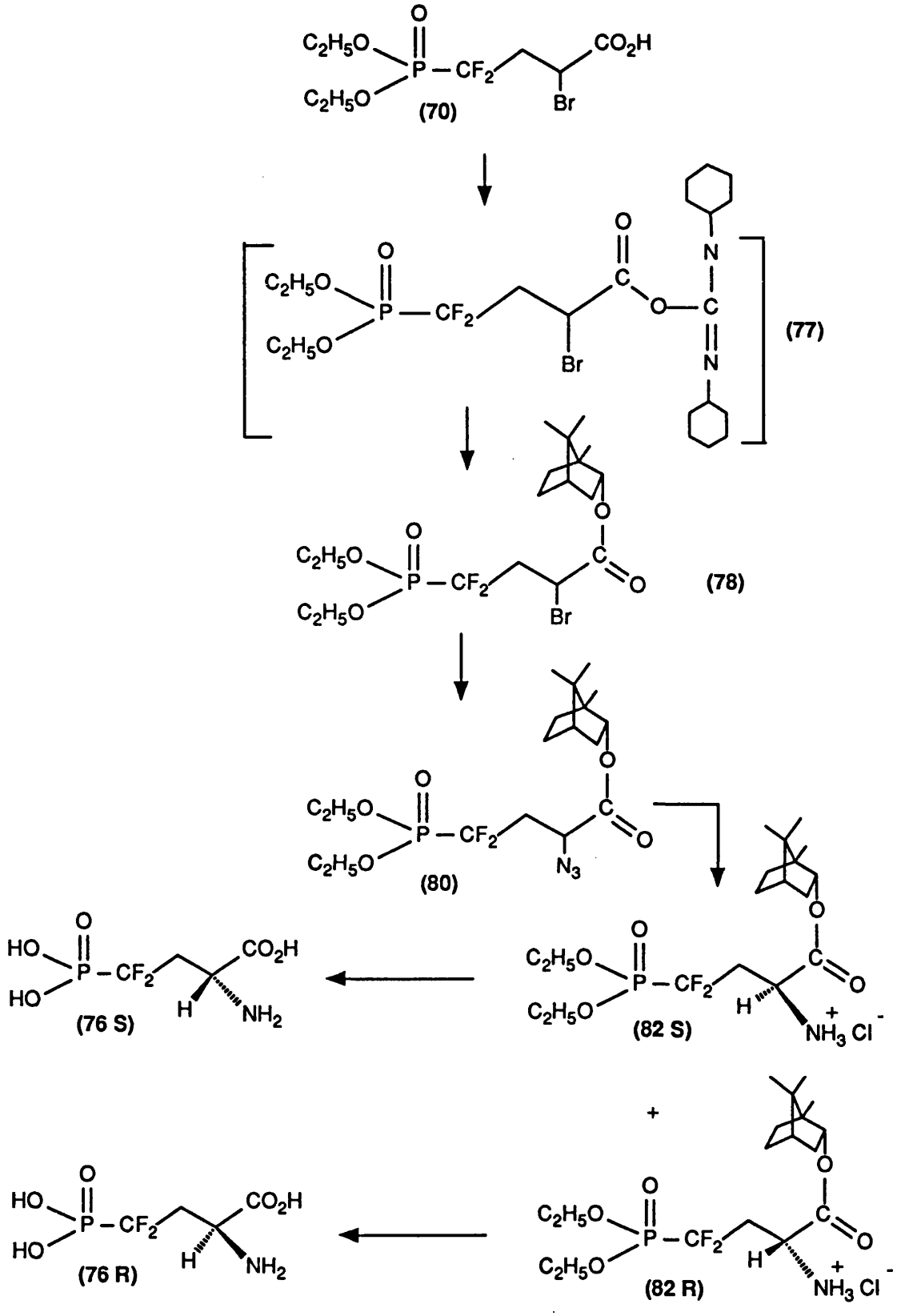
Scheme 23

Aminophosphonic acids are becoming increasingly important as analogues of natural amino acids. Thus several syntheses involving a variety of different strategies have emerged recently[68]. In order to explore the potential biological activity of the amino acid phosphonates, the synthesis of [4,4-difluoro-4-(dihydroxyphosphinyl) -2-amino] butanoic acid (76), an analogue of phosphoserine, was also a target of interest in this project. The strategy employed in the preparation of phosphono amino-acid (76) is outlined in Scheme 24.



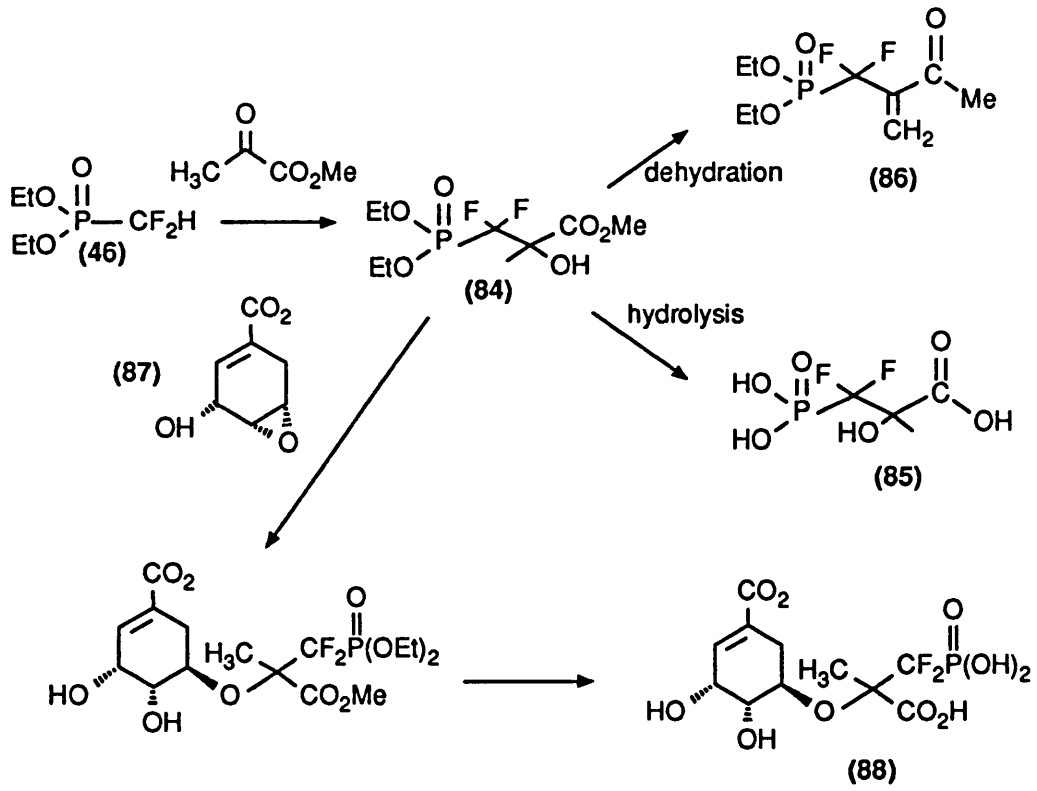
Scheme 24

Compound (76) is, however, racemic as shown. In order to isolate each enantiomer, the synthesis of [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (76) followed an alternative strategy, outlined in Scheme 25.



Scheme 25

Methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-hydroxy-2-methyl propionate (**84**), was another target compound in this project (**Scheme 26**). This compound had two potential uses in the shikimate pathway. Firstly, dehydration would lead to compound (**86**), an analogue of PEP. Secondly, it had potential use in the nucleophilic ring opening of the available epoxide (**87**)[10], leading to compound (**88**), an analogue of the tetrahedral intermediate (**13**)(**Figure 20**). Methyl [3,3-difluoro-3-(diethoxy phosphinyl)-2-hydroxy-2-methyl] propionate (**84**) will be the precursor of the compounds [3,3-difluoro-3-(dihydroxyphosphinyl)-2-hydroxy-2-methyl] propionic acid (**85**) and methyl 2-[(diethoxyphosphinyl) difluoromethyl] propenoate (**86**).



Scheme 26

The dehydration of compound (84) would result in the same compound already referred to by Phillon et al.[56], 2-[(dihydroxyphosphinyl) difluoromethyl] propenoic acid, (**Figure 22**), but using a different pathway. According to the authors, this compound demonstrated time-dependent inhibition for the enzyme EPSP synthase, when it was coupled with shikimate 3-phosphate.

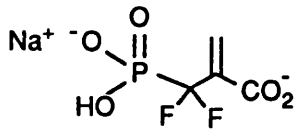


Figure 22

2.2 Synthesis of Methyl 2-[2',2'-difluoroethyl-2'-(diethoxyphosphinyl)] propenoate (67) and Methyl [2',2'-difluoroethyl-2'-diethoxy phosphinyl] propenoic acid (68)

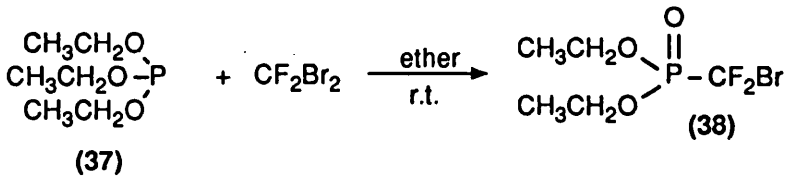
2.2.1 Preparation of Diethyl bromodifluoromethanephosphonate (38)

Diethyl bromodifluoromethylphosphonate (**38**), was prepared following the method described by Burton and Flynn[5]. They reported that dibromofluoromethane undergoes a facile Michaelis-Arbuzov reaction with alkyl phosphites in triglyme (TG) or diethyl ether (Et_2O) at 25-50°C to give the appropriate bromodifluoromethyl phosphonate esters.

The reaction was carried out by the addition of dibromofluoro methane to a stirred solution of triethyl phosphite in dry diethyl ether under nitrogen at room temperature (**Scheme 27**). The reaction took some time to initiate but, once started, the reaction was very exothermic, and had to be cooled immediately to avoid a build up of pressure inside the reaction vessel. When the procedure was carried out at 0°C, the reaction did not occur. Once the reaction started, it did not take more than 5 minutes to complete. The reaction mixture was concentrated and purified using column

chromatography, to give the desired product in 90% yield.

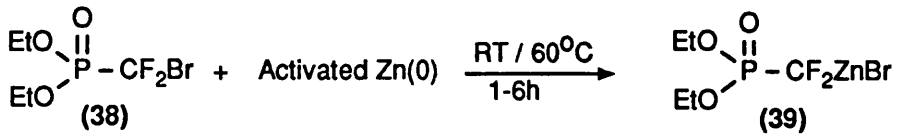
Another important point for this reaction is the quantity of solvent that should be used. The reaction was more effective when it was carried out without solvent, but since it is very exothermic, solvent was necessary to absorb the heat of the reaction. However, when the quantity of solvent was increased, the starting time for the reaction increased as well. Since the CF_2Br_2 is very volatile (b.p. 23°C), the yield of product decreased, resulting in unreacted triethyl phosphite.



Scheme 27

2.2.2 Preparation of [(Diethoxyphosphinyl) difluoromethyl] zinc bromide (39)

[Diethoxyphosphonyl) difluoromethyl] zinc bromide (**39**) was prepared according to the procedure of Burton *et al.*[51] . The reaction was carried out by treating dialkyl bromodifluoromethylphosphonate (**38**) with 1.1 equivalent of activated zinc dust in THF, under nitrogen, at r.t., which afforded the compound (**39**) as colourless oil in 99% yield (**Scheme 28**).



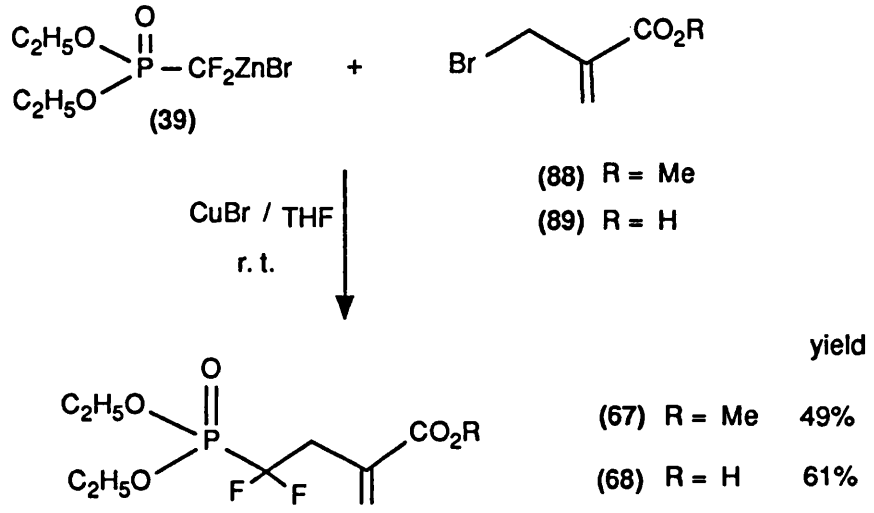
Scheme 28

The reaction was very clean and, therefore, the purification of compound (39) was not necessary prior to further reactions.

In a more recent publication Burton *et al.*[7] referred to the preparation of [(diethoxyphosphinyl) difluoromethyl] zinc bromide, with some modification of the process described above[51]. The diethyl bromodifluoromethanephosphonate was added slowly in a cooled ice bath, in order to avoid a vigorous exothermic reaction.

2.2.3 Coupling Reaction

Targets (67) and (68) were prepared following the method described by Burton *et al.*[7], for the reactions with allyl halides. The reaction was carried out by treating [(diethoxyphosphinyl) difluoromethyl] zinc bromide (39) in dry THF at room temperature with methyl 2-(bromomethyl) acrylate (88), and methyl 2-bromomethyl acrylic acid (89) under nitrogen, to give the compounds (67) and (68), both as a pale oil, after column chromatography, in 49% and 61% yield, respectively (**Scheme 29**).



Scheme 29

Mass spectral analysis of compounds (**67**) and (**68**) indicated the elemental composition of $C_{10}H_{17}F_2O_5P$ and $C_9H_{15}F_2O_5P$ (MH^+ 287, 100%; and MH^+ 273, 100% respectively). The infra-red spectrum (liquid film) indicated the presence of a vinyl group (ν_{max} 1660 cm^{-1} for compound **67** and ν_{max} 1634 cm^{-1} for compound **68**). The ^1H NMR data for compound (**67**) and (**68**) is summarised in **Table II.**

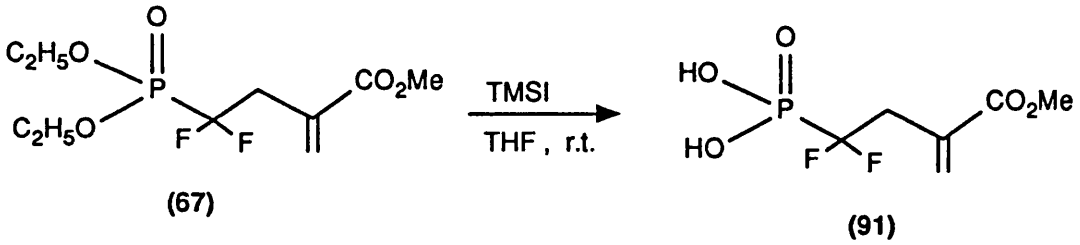
Table II

compound	chemical shift (δ)		coupling constants (Hz)	
	3 - H (vinylic)	1' - H	$J_{1',F}$	$J_{1',P}$
$(EtO)_2PCF_2CH_2CCO_2Me$ (**67**)	5.98 6.47	3.17	19.64	4.76
$(EtO)_2PCF_2CH_2CCO_2H$ (**68**)	5.98 6.60	3.17	19.54	4.88
$(HO)_2PCF_2CH_2CCO_2Me$ (**91**)	5.81 6.29	3.12	19.90	3.85

The resonances due to the vinylic protons were two singlets at 5.89 ppm and 6.64 ppm for compounds (**67**), and at 5.89 and 6.60 for compound (**68**), which is characteristic for vinylic protons. The triplet of doublets at 3.17 ppm for both compounds (**67**) and (**68**), were assigned for the allylic protons, resulting from the coupling of the protons with fluorine and phosphorus ($J_{1',P}$= 4.76 Hz and $J_{1',F}$= 19.64

Hz for compound **67**, and $J_{1',P}$= 4.88 Hz and $J_{1',F}$= 19.54 Hz for compound **68**). The singlet at δ= 3.79 ppm in the ^{1}H, and at 52.02 ppm in the ^{13}C spectrum of compound (**67**), confirmed the presence of an OMe group.

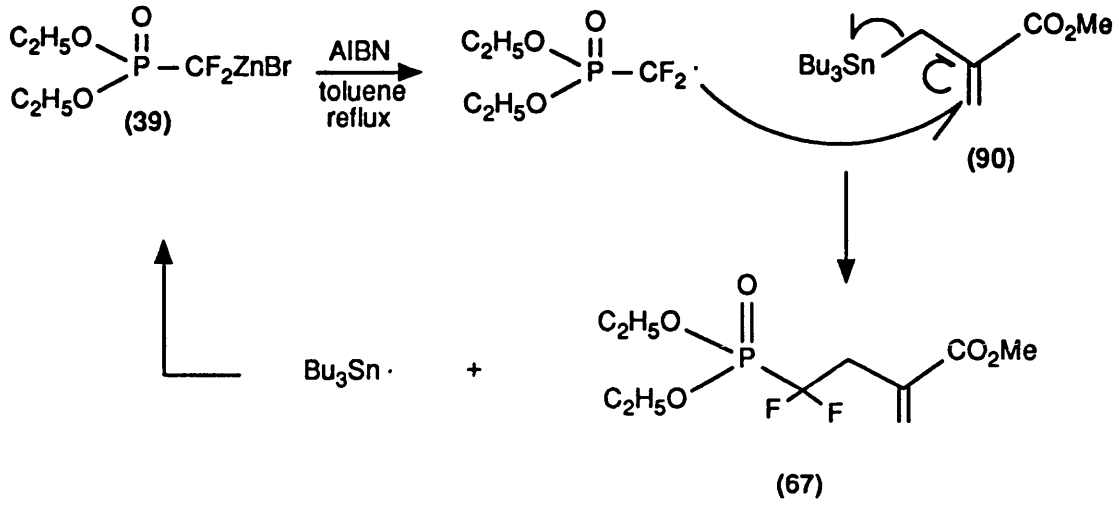
The ester (**67**) was hydrolysed with TMSI, in order to afford the corresponding 2-[2',2'difluoroethyl-2'-(dihydroxyphosphinyl) propenoic acid (**69**) but, unfortunately, the methyl ester was not hydrolysed, yielding the compound (**91**) instead (**Scheme 36**). The OMe group was evident from NMR data, giving rise to a singlet at δ= 3. 63ppm in the ^{1}H NMR spectrum and a singlet at δ= 53.55ppm in the ^{13}C NMR spectrum. The molecular formula was confirmed by the mass spectrum m/z(-ve FAB) 229(MH^-, 100%).



Scheme 36

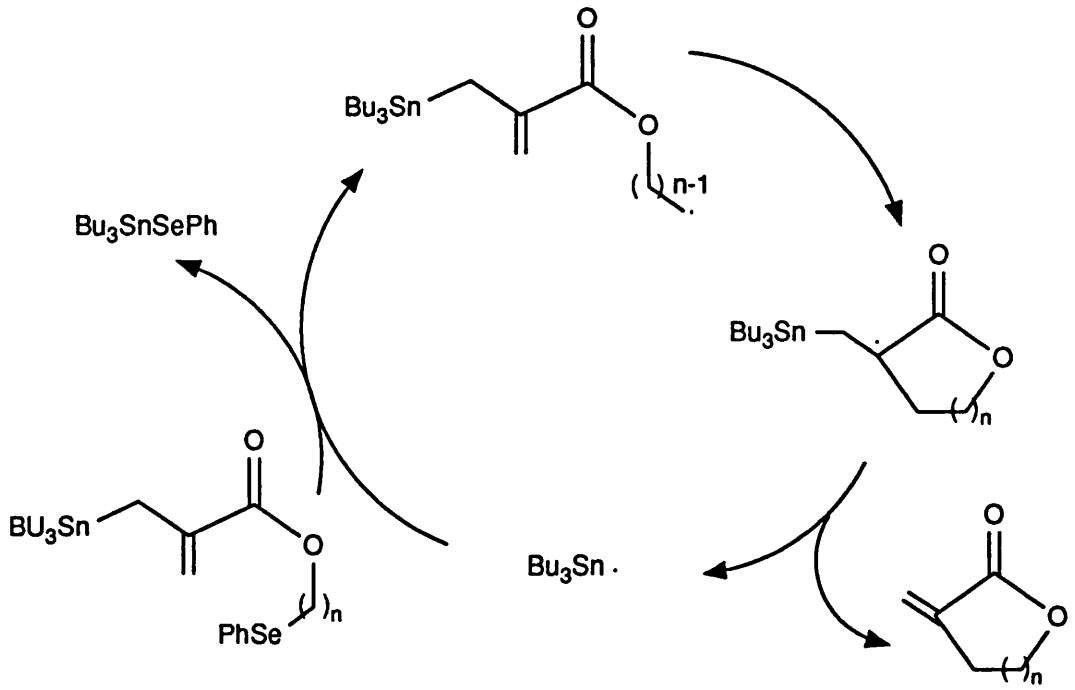
Due to the incomplete hydrolysis of compound (**67**), the synthesis of compound (**69**) was attempted by an alternative route, through hydrolysis of compound (**68**).

Compound (**67**) was also synthesized by radical addition of [(diethoxyphosphinyl) difluoromethyl] zinc bromide (**39**). The organometallic compound (**39**), containing methyl tributyltin methacrylate ($Bu_3SnCH_2C(CH_2)CO_2Me$) and a catalytic amount of azobis(isobutyronitrile)(AIBN), was refluxed in dry toluene for 12 hours, and then cooled to room temperature; after the solvent was removed column chromatography furnished compound (**67**) in low yield (10%)(**Scheme 30**).

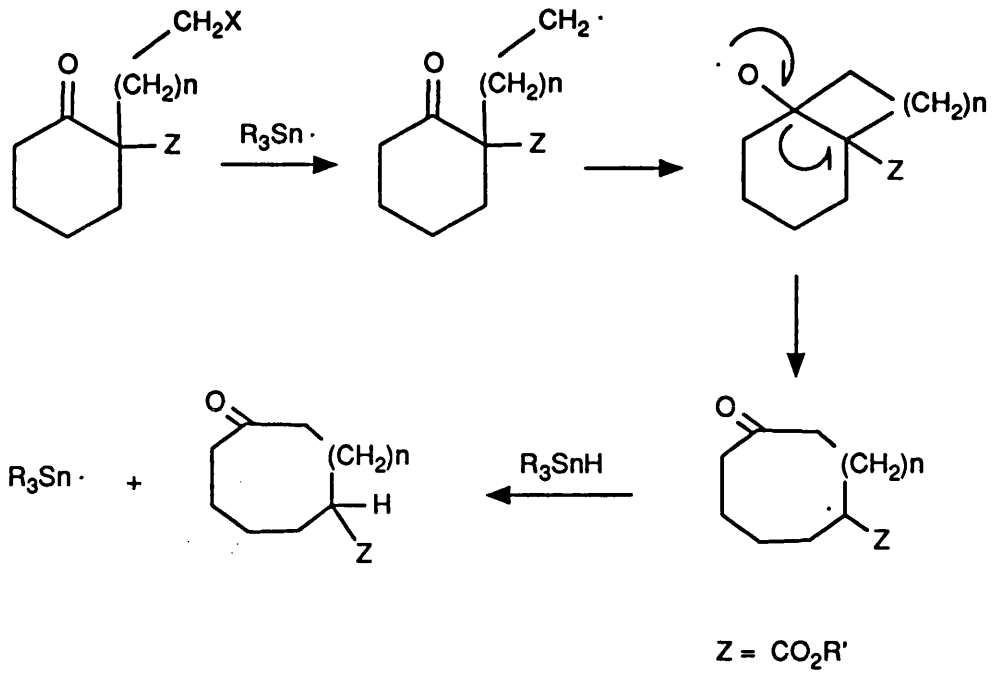


Scheme 30

Radical reactions using azobis(isobutyronitrile)(AIBN), as catalyst and Bu_3SnH, were referred to by Baldwin *et al.* in many reactions, including intramolecular S_H2' macrocyclisation[69] (**Scheme 31**), and carbocyclic ring expansion[70] (**Scheme 32**).

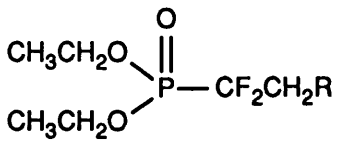


Scheme 31 - Typical procedure for macrocyclisation



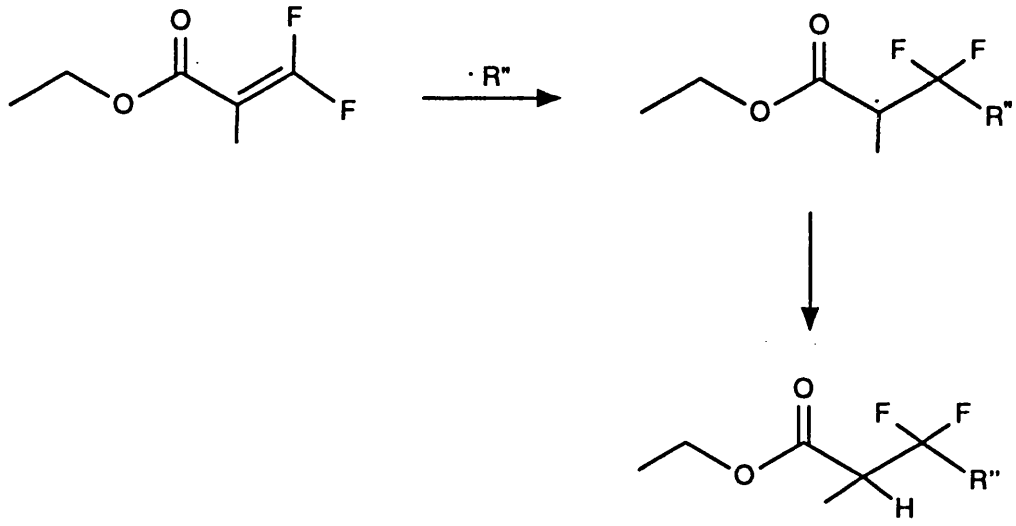
Scheme 32 - Carboxylic ring expansion via radical chain processes

Martin *et al.*[8] referred to the synthesis of 1,1-difluoroalkylphosphonates (**49**) (**Scheme 12**, section 1.7), using AIBN and tributyltin hydride (Bu_3SnH).



(49)

Bumgardner *et al.*[71] studied the radical additions to β,β-difluoroacrylates (**Scheme 33**). They used 1 mol% of AIBN and 1 mol% of benzoylperoxide (BPO).

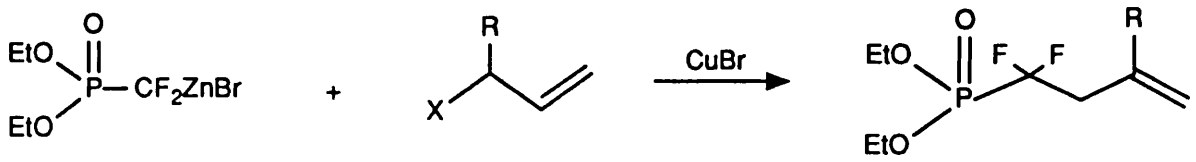


Scheme 33

Although there are many well known radical reactions using AIBN and Bu_3SnH, no reaction using difluoroalkyl zinc bromide and methyl tributyltin metacrylate has been reported.

However, due to the poor yield offered by this method, the choice for the preparation of compound (**67**) was best achieved by the method described by Burton *et al.*[7].

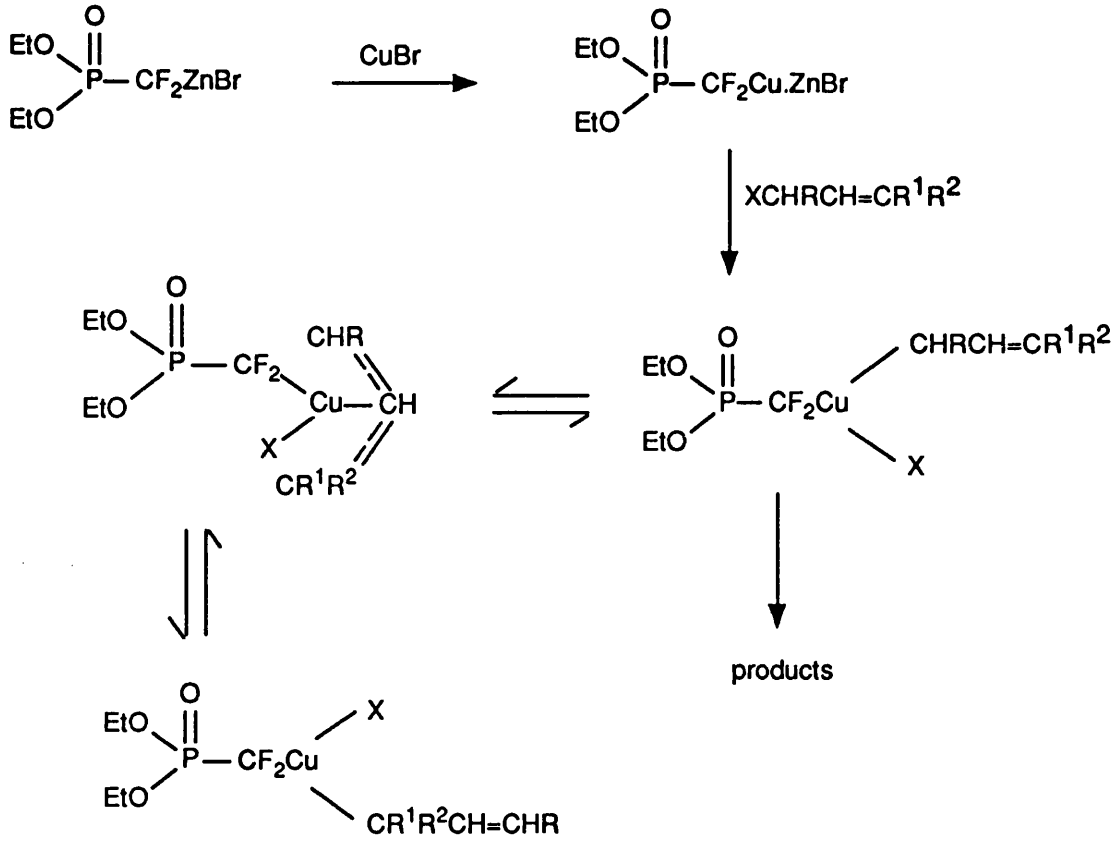
According to the literature[72], the reaction of [(diethoxyphosphinyl) difluoromethyl] zinc bromide with allylic halides, catalyzed by CuBr, gave the corresponding 1,1-difluoro-3-alkenyl phosphonates, which resulted from displacement of the halogen by the organozinc reagent (**Scheme 34**).



Allylic halide	Product	Yield
$BrCH_2CH=CH_2$	$(EtO)_2P(O)CF_2CH_2CH=CH_2$	47%
$BrCH_2CBr=CH_2$	$(EtO)_2P(O)CF_2CH_2CHBr=CH_2$	52%
$BrCH_2CH=CHCH_3$	$(EtO)_2P(O)CF_2CH_2CH=CHCH_3$	49%
$ClCH_2CH=CHPh$	$(EtO)_2P(O)CF_2CH_2CH=CHPh$	55%

Scheme 34

A possible mechanism to explain the organocopper chemistry consists of an oxidative addition following a reductive elimination. Symmetrical (π-allyl) copper(III) complexes have been postulated as reaction intermediates. Oxidative addition of an allylic halide to an intermediate cuprous complex would yield a d^{10} allylic complex, which would be expected to exist in a number of η^1 and η^3 isomeric structures, as ilustrated in **Scheme 35**. Reductive elimination of two organic ligands from an η^1 isomer would result in the observed products.



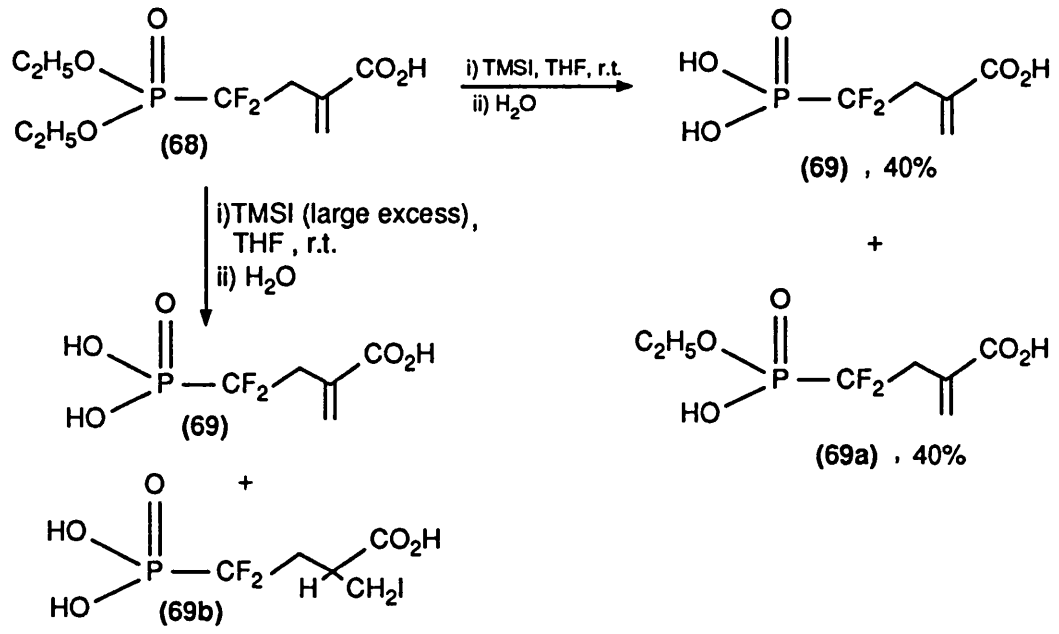
Scheme 35

2.3 Synthesis of 2-[2',2'-Difluoroethyl-2'-(dihydroxyphosphinyl)] propenoic acid (69)

Treatment of 2-[2',2'-difluoroethyl-2'-(diethoxyphosphinyl] propenoic acid (68) with iodotrimethylsilane (TMSI) resulted in the corresponding bis trimethyl silyl ester, which in turn was hydrolysed to afford the expected parent phosphonic acid, 2-[2',2'-difluoroethyl -2'-(dihydroxyphosphinyl)] propenoic acid (69). However, the partially hydrolysed compound (69a), was also detected in the reaction mixture. These acids (69 and 69a, 1:1 mixture, by mass), were separated by column chromatography. Use of a large excess of TMSI, in order to avoid the formation of the partially

hydrolysed compound (**69a**), resulted in the attack of TMSI at the vinyl group of compound (**68**), generating the by-product (**69b**), which could not be separated from compound (**69**) by column chromatography (**Scheme 37**). If the reaction of compound (**68**) with TMSI was carried out without solvent, only product (**69b**) was observed.

The presence of an ethyl group in the compound (**69a**) was indicated in the ^1H NMR by the presence of resonances due to 3-H of the ethyl group as a triplet at 1.39 ppm ($J_{H,H}$= 7.05 Hz), and to 2-H of the ethyl group as a multiplet at 3.94 ppm, which was confirmed by mass spectrum m/z(-ve FAB) 243(MH^-, 100%). For the product (**69b**), the resonances due to the CH_2I protons appeared as a multiplet at 1.30 ppm. The mass spectrum (-ve FAB) showed a mass ion at m/z 343(MH^-, 30%) confirming the formation of the undesired product (**69b**). In the IR, the band around 1630 cm^{-1}, which is characteristic of the double bond, was not present. Treatment of compound (**69b**) with LDA, BuLi, and DBU, was tried in order to obtain the compound (**69**) but, unfortunately the reaction proceeded with the formation of too many unseparable by-products.



Scheme 37

The elemental composition of 2-[2',2'-difluoroethyl-2'-(dihydroxyphosphinyl)] propenoic acid (**69**) as $C_5H_7F_2O_5P$ was indicated by mass spectrum which showed a molecular ion at m/z(-ve FAB) 215(MH^-, 35%). The presence of vinyl groups was evident from infra-red spectrum, which contained a band at 1630 cm^{-1}.

The ^{1}H-NMR spectrum for this compound showed resonances due to the α-vinylic protons (two singlets) at 5.89 and 6.34ppm. The β-allylic protons appeared as a multiplet (dt), due to the coupling with fluorine and phosphorus ($J_{H,P}$= 2.47 Hz, $J_{H,F}$= 20.4 Hz).

2-[2',2'-Difluoroethyl -2'-(dihydroxyphosphinyl)] propenoic acid (**69**) is an analogue of 2-(phosphonomethyl) acrylic acid (**Figure 23**), which was prepared by Radzicka *et al.*[67] as an effective inhibitor of prolidase. Thus compound (**69**) could be not only an inhibitor for the shikimic acid pathway, but also a potential inhibitor of prolidase.

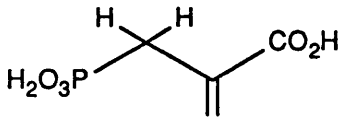
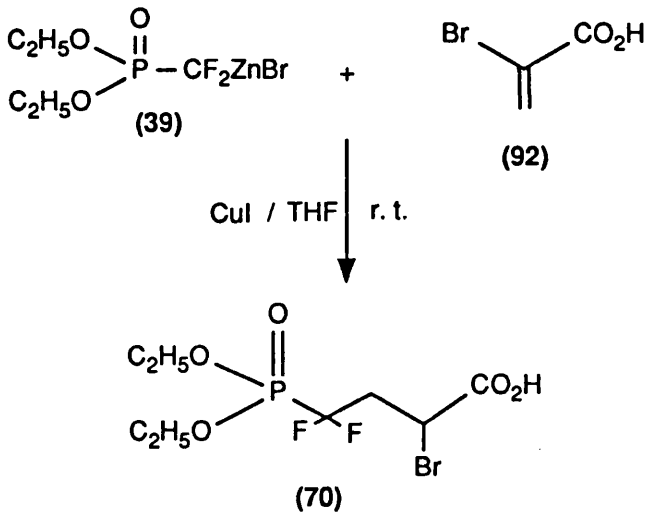


Figure 23 - 2-(Phosphonomethyl) acrylic acid

2.4 Synthesis of [4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (70)

[4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**) was prepared using the method described by Burton *et al.*[7]. [(Diethoxyphosphinyl) difluoromethyl] zinc bromide (**39**), in dry THF at room temperature, was reacted with 2-bromoacrylic acid (**92**) under N_2 in the presence of a catalytic amount of CuBr (**Scheme 38**) to give the compound (**70**) in 33% yield.



Scheme 38

Analysis of compound (**70**) indicated the elemental composition $C_8H_{14}BrF_2O_5P$ which was in agreement with the mass spectrum (MH^+ 339,341 98%). The infra-red data (liquid film) indicated the presence of a bromine group (C-Br 750-500)(ν_{max} 704 cm^{-1}).

The ^1H NMR for compound (**70**) showed a dd at 4.55 ppm ($J_{2,3a}$= 4.39 Hz, $J_{2,3b}$= 9.28 Hz), assigned as CH(Br). The resonances due to non-equivalent protons, 3a and 3b, next to the chiral centre of the compound (**70**), was complicated, appearing as a multiplet at 3.09-3.34 ppm. However, the assignment of the coupling constants for the protons 3a and 3b was taken from ^{19}F and ^{31}P (coupled and decoupled). Since the two fluorines are non-equivalent, a typical AB pattern is always observed in the ^{19}F NMR spectrum. For example, figure **23a** illustrates an AB splitting pattern for [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**). The coupling constant between the two fluorine atoms was 301.7 Hz, with a fluorine-phosphorus coupling constant of 105.2 Hz. The coupling constants of the two vicinal hydrogens

with fluorine were observed at 25.4 Hz and 12.7 Hz. The phosphorus-fluorine coupling constant, $J_{P,F}$= 104 Hz, was observed in the ^{31}P NMR (decoupled), appearing as a triplet. ^{31}P NMR (coupled) allowed the $J_{3a,P}$= $J_{3b,P}$= 4.03 Hz to be measured.

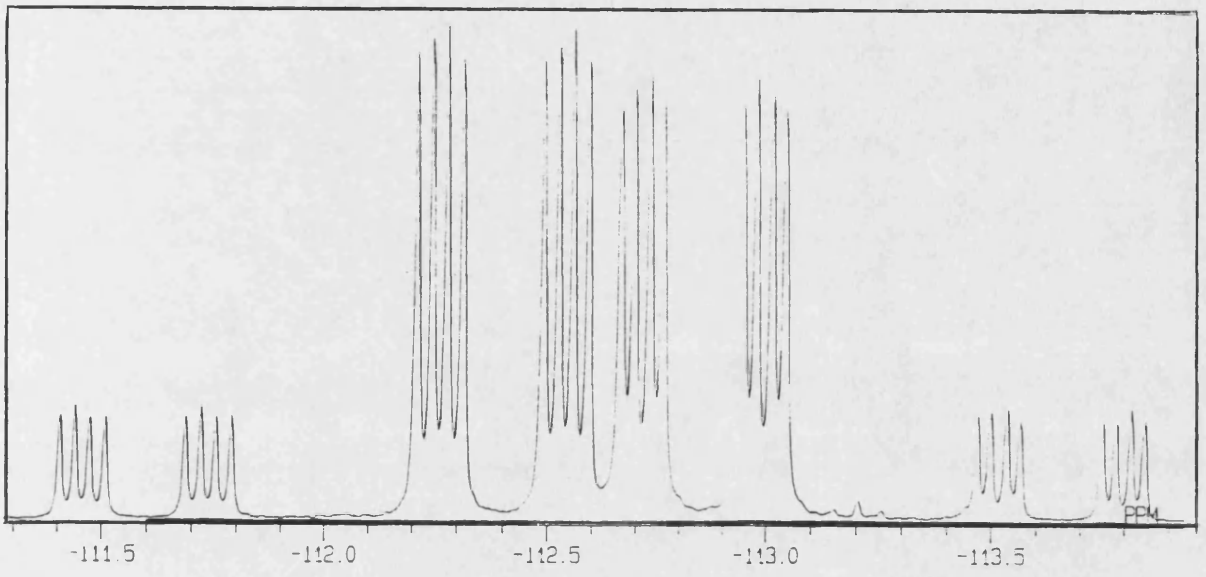
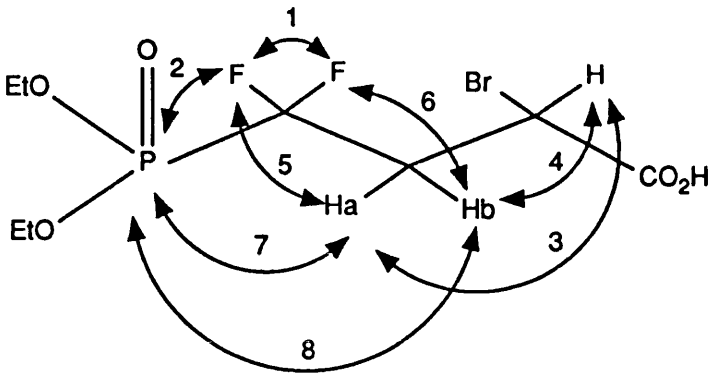


Figure 23a - Observed ^{19}F NMR spectrum for compound **(70)**

Homonuclear decoupling experiments were used to determine the coupling interaction of the geminal protons. Irradiation at the resonance due to 2-H simplified each 3-H resonance to 16 lines (dddd), and the coupling interaction of the geminal protons, could be determined ($J_{3a,3b}$= 26.43 Hz).

The coupling interactions are summarized in **Figure 24**.



1 $J_{F,F}$= 301.7 Hz

2 $J_{P,F}$= 105.2 Hz

3 $J_{2,3a}$= 9.28 Hz

4 $J_{2,3b}$= 4.39 Hz

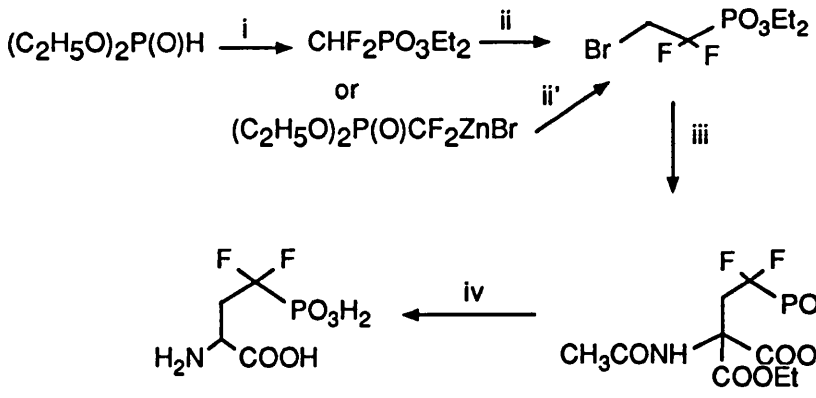
5 $J_{3a,F}$= 12.7 Hz

6 $J_{3b,F}$= 25.4 Hz

7, 8 $J_{3a,P}$=$J_{3b,P}$= 4.03 Hz

Figure 24

Another alternative method to synthesize the compound (**70**) was attempted using the general preparative method of Bigge *et al.* [55] for the synthesis of aminoacids (**Scheme 39**).



Reagents and conditions:

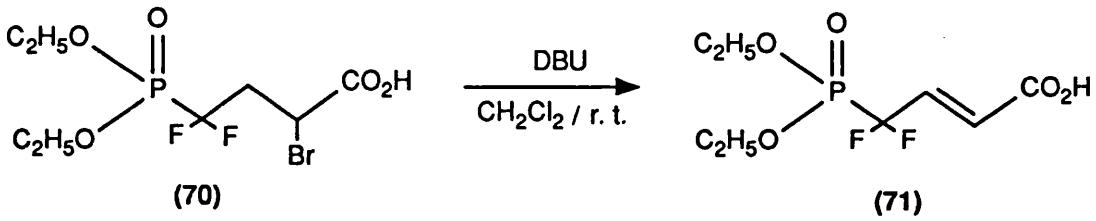
i) NaH, CHF_2Cl, THF; ii) LDA, THF, CH_2Br_2; ii') CuBr, THF, CH_2Br_2;
iii) $NaC(NHAc)(CO_2Et)_2$, EtOH, Reflux; iv) 6M HCl, reflux.

Scheme 39

Unfortunately, the reaction of $(C_2H_5O)_2P(O)CF_2H$ or $(C_2H_5O)_2P(O)CF_2ZnBr$ with dibromomethane did not succeed.

2.5 Synthesis of E-[4,4-Difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid (71)

The preparation of compound (**71**) was achieved by the treatment of compound (**70**) in CH_2Cl_2, with 2 equivalents of DBU at room temperature (**Scheme 40**).



Scheme 40

As can be seen above, the E-isomer was formed. This might be the result of elimination from Newman conformation A, rather than B (**Figure 25**), although there are mechanisms for the conversion of the Z-isomer into the E-isomer.

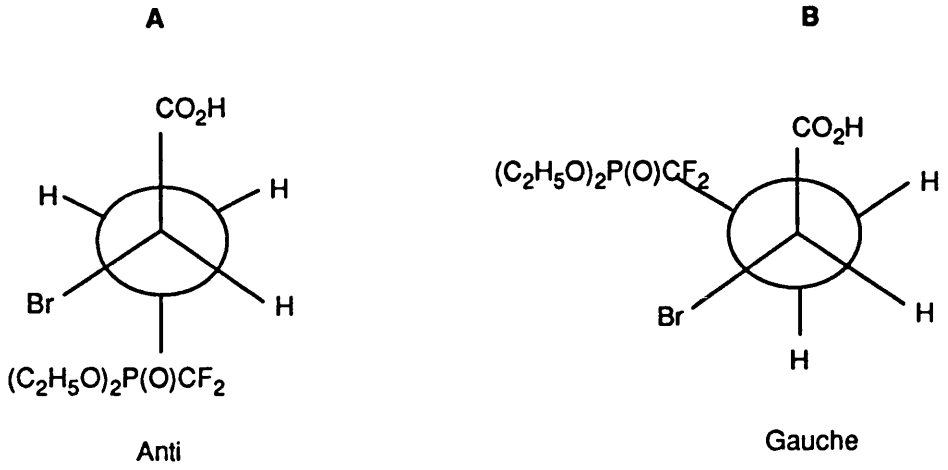


Figure 25

In the gauche conformation the three large groups (Br, CO_2H, and $(C_2H_5O)_2P(O)CF_2$) are near each other, resulting in a steric strain and less stable configuration.

The elemental composition $C_8H_{13}O_5F_2P$ was indicated by the mass spectrum and confirmed by accurate mass measurement (MH^+ 259.0547, 100%). The strong band in the IR (liquid film) at 1641 cm^{-1} indicated the presence of the double bond.

^1H NMR spectrum also helped in confirming the structure of the compound. The resonances due to the proton in the 2α position appeared as doublet of quartets centered at 6.40 ppm, resulting from the coupling with 3β proton and with fluorine and phosphorus ($J_{2\alpha,3\beta}$ = 15.8 Hz, $J_{2\alpha,F}$ = 5.31 Hz, $J_{2\alpha,P}$ = 2.57 Hz). The 3β proton appeared as a multiplet (dtd) centered at 6.92 ppm ($J_{3\beta,2\alpha}$ = 15.8 Hz, $J_{3\beta,F}$ = 12.6 Hz, $J_{3\beta,P}$ = 1.95 Hz).

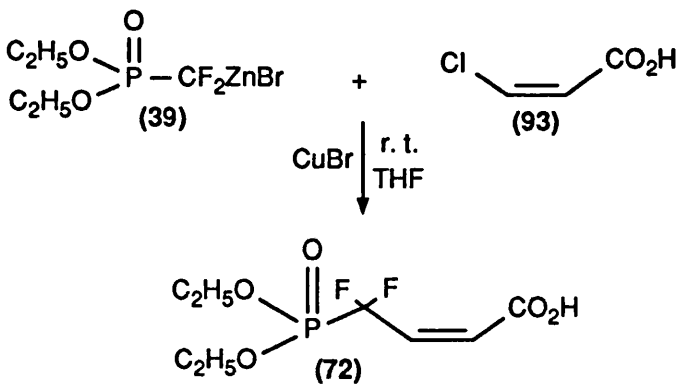
The n.O.e experiments confirmed the E-configuration of the compound (**71**). When the irradiation was carried out at the position of the 2α proton (6.40 ppm), the resonance frequence due to the 3β proton was not affected. Irradiation at the resonance due to the 3β proton, also did not affect the resonance signal due to the 2α proton. Since n.O.e effect is only noticeable over short distances, generally 2-4 Å, it is clear

that the two protons (2α and 3β) are in the trans-position.

The ^1H NMR spectroscopic coupling constant of 15.8 Hz between the vinylic hydrogens (2α and 3β) also establishes the E stereochemistry[110].

2.6 Synthesis of Z-[4,4-Difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid (72)

Z[4,4-Difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid (**72**), which is an isomer of the compound (**71**), was synthesized using the same preparative method described by Burton *et al.*[7]. The [(diethoxyphosphinyl) difluoromethyl] zinc bromide in dry THF, at room temperature, was reacted with cis-3-chloroacrylic acid (**93**) under N_2, in the presence of catalytic amount of cuprous bromide, to give the compound (**72**) in 20% yield (**Scheme 41**).



Scheme 41

The compound (**72**) has the same Z configuration as the starting cis-3-chloroacrylic acid. This configuration was observed from the comparative NMR analysis of compound (**72**) and (**71**). The chemical shifts and coupling constants for both compounds are in **Table III.**

Table III

Compound	2 α vinylic proton		3 β vinylic proton	
	Chemical Shift	Coupling Constant (Hz)	Chemical Shift	Coupling Constant (Hz)
$(EtO)_2\overset{O}{\overset{\|}{P}}CF_2CH=CHCO_2H$ E - **(71)**	6.40	15.8	6.92	15.9
$(EtO)_2\overset{O}{\overset{\|}{P}}CF_2CH=CHCO_2H$ Z - **(72)**	6.37	12.9	6.01	12.8

The β vinylic hydrogen of the compound (**72**) was shifted downfield to δ 6.9 ppm. The smaller coupling constant for the compound (**72**) indicated the Z configuration.

Analysis of compound (**72**) indicated the elemental composition of $C_8H_{13}O_5F_2P$, which was in agreement with the mass spectrum (MH^+ 259, 100%), and confirmed by accurate mass measurement (MH^+ 259.0547, $C_8H_{13}O_5F_2P$ requires 259.0547). The strong band at 1657 cm^{-1} in the IR (liquid film) indicated the presence of the double bond in the compound.

The resonances due to the protons in the 2α position appeared as a doublet of quartets centered at 6.37 ppm, resulting from the coupling with 3β proton and with fluorine and phosphorus ($J_{2\alpha,3\beta}$ = 12.9 Hz, $J_{2\alpha,F}$ = 2.47 Hz, $J_{2\alpha,P}$ = 2.47 Hz). The 3β proton appeared as a multiplet (dtd), centered at 6.01 ppm ($J_{3\beta,2\alpha}$ = 12.8 Hz, $J_{3\beta,F}$ = 12.7 Hz, $J_{3\beta,P}$ = 1.94 Hz).

The n.O.e experiments were used to confirm the Z configuration of the compound (**72**). When the irradiation was carried out at 2-H (6.37 ppm), the integration for 3-H increased (42%). Irradiation at the position for the 3-H proton (6.01 ppm) increased the integration for 2-H (42%). This n.O.e effect observed in compound (**72**)

indicated a short distance, 2-4 Å,, between the 2-H and 3-H protons, which confirmed the protons (2-H and 3-H) in the cis-position.

A proposed mechanism for the formation of this product is illustrated in **Figure 26**[111].

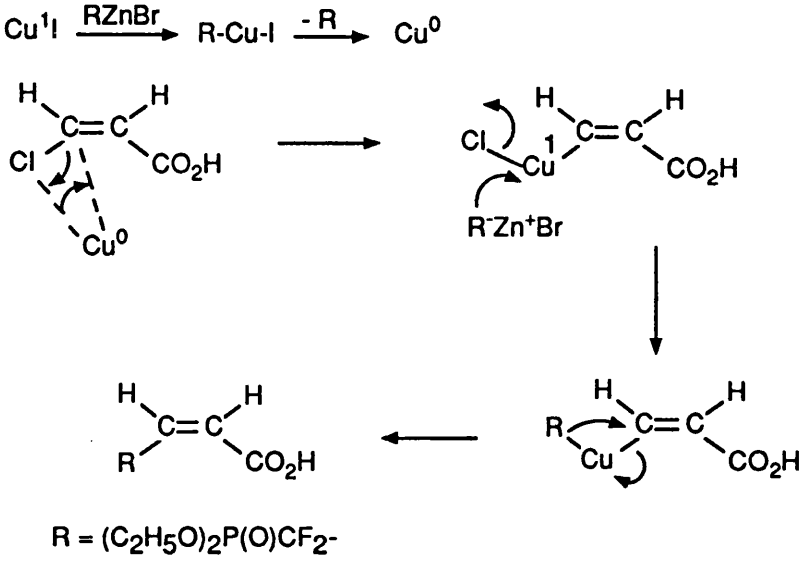


Figure 26 - Proposed mechanism for the preparation of 4,4-difluoro-4-(diethyl phosphono)-Z-but-2-enoic acid (72)

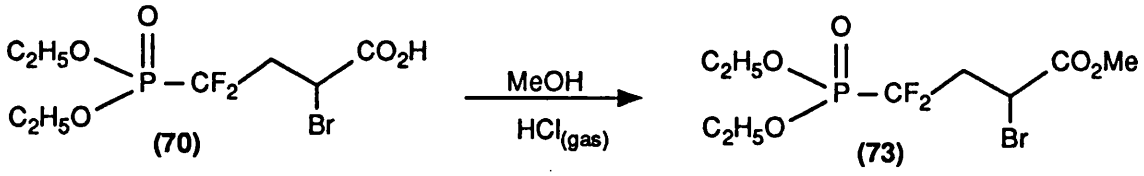
2.7 Synthesis of [4,4-Difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (81)

2.7.1 Preparation of Methyl [4,4-difluoro-4-(diethoxyphospinyl)-2-bromo] butanoate (73)

Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoate (**73**) was prepared by esterification of [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**). The esterification of carboxylic acids is well documented, and there are many excellent methods for the transformation. Fischer and Speier[73] discovered, in 1895,

that esters result from simply heating a carboxylic acid in methanol or ethanol solution containing a small amount of mineral acid catalyst, and this procedure was therefore employed.

[4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**) reacts with methanol in the presence of $HCl_{(gas)}$, to give the desired ester product in 76% yield (**Scheme 42**).



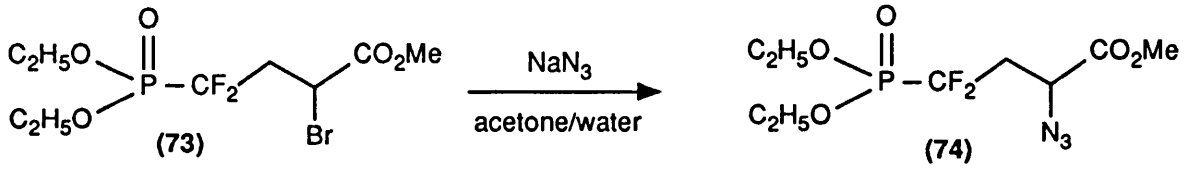
Scheme 42

The elemental composition $C_9H_{16}O_5F_2BrP$ was indicated by mass spectrum (MH^+ 353,355 100%), and also confirmed by accurate mass measurement (MH^+ 353.98663; $C_9H_{16}O_5F_2BrP$ requires 353.9872).

The ^1H NMR spectrum of the compound (**73**) was similar to compound (**70**). The resonances due to 2-H appeared as dd at 4.57 ppm ($J_{2,3a}$ = 4.12 Hz, $J_{2,3b}$ = 9.61 Hz). The ^{19}F NMR and ^{31P}P NMR (coupled and decoupled) gave the assignments of the coupling constants for the protons 3a and 3b. The ^{19}F NMR spectrum of compound (**73**) appeared as an AB pattern since the two fluorine atoms are diastereotopic. The coupling constant between the two fluorine atoms was 301.7 Hz, with a fluorine-phosphorus coupling constant of 103.5 Hz. The coupling constants of the two vicinal hydrogens with fluorine were observed at 22.5 Hz and 15.1 Hz. The phosphorus-fluorine coupling constant, $J_{P,F}$ = 104 Hz, was observed in the ^{31}P NMR (decoupled) appearing as a triplet. The singlet at 3.81ppm in the ^1H NMR confirmed the presence of methyl ester.

2.7.2 Azide displacement of the Bromine Group

The reaction of methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoate (**73**), with sodium azide in a polar solvent, proceeded successfully to give the azide product as a pale oil, after column chromatography, in 65% yield. The displacement proceeded with complete regiospecificity, resulting from the direct nucleophilic displacement of the bromide on the α-carbon by the azide anion. No displacement of fluorine occurred (**Scheme 43**).



Scheme 43

In the ^{1}H NMR the signal from the α proton moved upfield to 4.26-4.35 ppm where it overlapped with the signal from the methylene protons of the ethyl group. However, irradiation at the position corresponding to methyl protons of the ethyl group (1.40 ppm) simplified the methylene protons resonance, and allowed the couplings in the 2-H resonance to be measured ($J_{2,3a}$ = 4.27 Hz, $J_{2,3b}$ = 8.24 Hz). The $J_{H,F}$, $J_{H,P}$, $J_{F,F}$ and $J_{F,P}$ for 3-H was determined from ^{19}F NMR and ^{31}P NMR (coupled and decoupled) and, as it was expected, they exhibited values similar to compounds (**70**) and (**73**) ($J_{3a,F}$ = 11.6 Hz, $J_{3b,F}$ = 25.5 Hz, $J_{F,P}$ = 104 Hz, $J_{F,F}$ = 300.2 Hz, $J_{P,H}$ = 4.76 Hz). The two fluorine atoms are diastereotopic and a typical AB pattern was observed in the ^{19}F NMR spectrum.

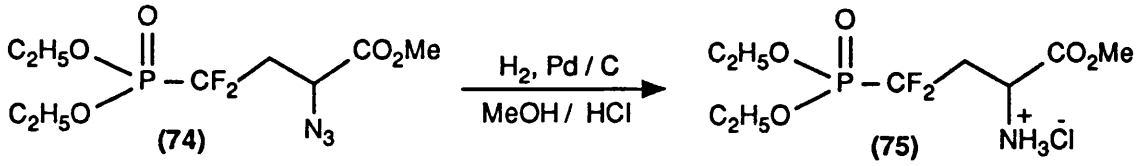
The elemental composition $C_9H_{16}O_5F_2N_3P$ was indicated by mass spectrum and confirmed by accurate mass measurement (MH^+ 316.0874, 100%). The azido group is

detected in the IR spectrum (liquid film), which showed a strong band at 2123 cm^{-1}.

2.7.3 Hydrogenation of the Azido Group

Reduction of alkyl azides, either by catalytic hydrogenation over palladium or by reaction with $LiAlH_4$, leads to the desired primary amine[73]. This is an important reaction in organic synthesis, especially in carbohydrate and nucleoside chemistry[74].

Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butanoate hydrochloride salt (**75**) was prepared by catalytic hydrogenation over 5% Pd/C using methanol as solvent. The presence of $HCl_{(conc.)}$ in the reaction mixture converted the resulting free amine in the more stable amine salt (**Scheme 44**).



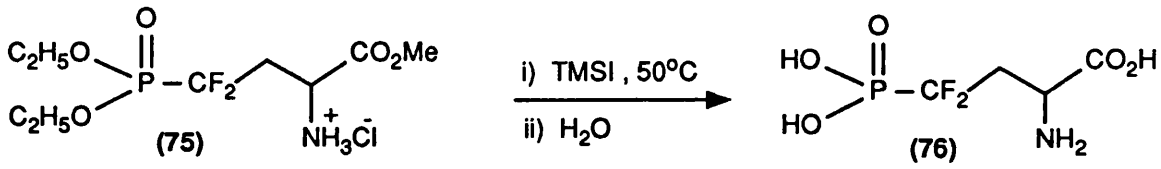
Scheme 44

The polar group, $NH_3^+Cl^-$, in the compound (**75**) reduced the shielding effect of the 2-H proton and lead to a higher chemical shift value, which then moved downfield at 4.68 ppm, appearing as a doublet of doublets ($J_{2,3a}$ = 4.03 Hz, $J_{2,3b}$ = 7.51 Hz). The elemental composition $C_9H_{18}O_5F_2NP.HCl$ was indicated by mass spectrum and confirmed by accurate mass measurement (MH^+ 290.0980, 100%).

2.7.4 Deprotection of (Diethoxyphosphinyl) and Methyl ester

The final stage in the synthesis of the target compound, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**76**), was the conversion of the ethyl and methyl groups to hydroxyl groups, and of the ammonium salt to the free amine.

Reaction of methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butanoate hydrochloride salt (**75**) with TMSI at 50°C followed by hydrolysis in water effected the cleavage of the ethyl and methyl groups and removal of the HCl, affording the free acid and free amine as a white solid in 82% yield (**Scheme 45**).



Scheme 45

The α-amino phosphonic acid, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**76**), is an analogue of phosphoserine. This analogue, although racemic, was therefore expected to have also an important biological activity, and it is of interest for biological testing.

The elemental composition $C_4H_8F_2NO_5P$ was indicated by mass spectrum and confirmed by accurate mass measurement, m/z (-ve FAB) 218 (MH^-, 75%). The strong band in the IR at 2527 cm^{-1} (possibly zwitterionic form) indicated the presence of the amine group.

The ^1H NMR data for this compound was less informative. The resonances due to 2-H were broad doublets at 4.32 ppm (J= 6.77 ppm), while those due to 3-H appeared as unresolved multiplets centered at 2.72 ppm. However, since the resonances due to 2-H and 3-H protons are similar to the previous compound (**75**), it helped in

elucidating the structure of compound (**76**), although full assignments for the coupling constants could not be done.

2.7.5 Coupling Reaction of [4,4-Difuoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid with [(1S)-endo]-(-)-Borneol.

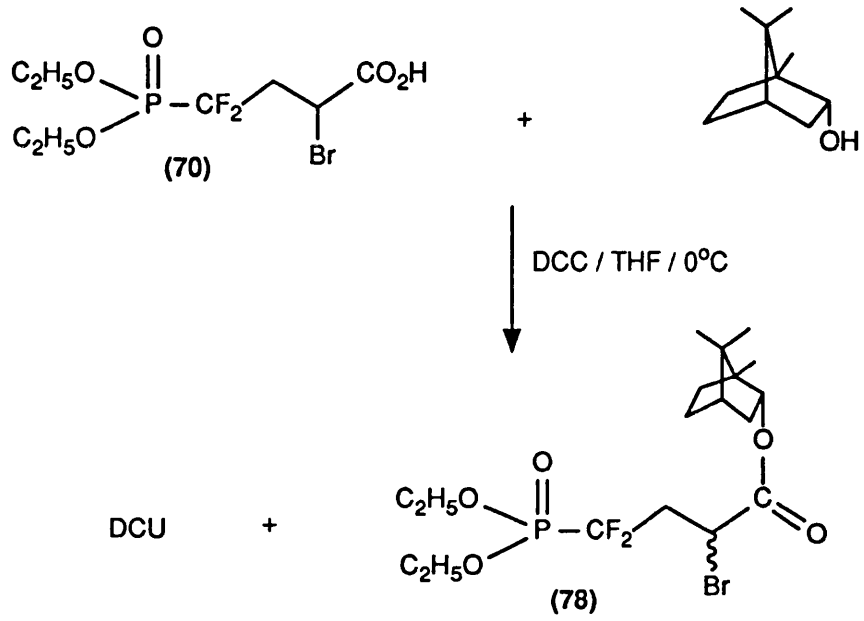
It was now necessary to explore the possible resolution of racemic (**76**) into its enantiomers.

[4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**) was coupled with [(1S)-endo]-(-)-borneol in dry THF. Dicyclohexyl carbodiimide was used to activate the carboxyl group of the compound (**70**): the carbodiimide method was introduced to peptide synthesis by Sheeham and Hess in 1955[75], and later was extended for the preparation of esters. DCC reacts with the carboxyl group of the compound (**70**) to form the O-acylisourea intermediate (**77**), a highly activated acylating agent, which was expected to react with [(1S)-endo]-(-)-borneol, yielding a mixture of diastereoisomers (**78**) (**Scheme 46**).

The intramolecular reaction of the intermediate (**77**) was a competing, unwanted side-reaction leading to the formation of the N-acylurea, thus causing problems in the synthesis of compound (**78**) due to difficulty of purification, and also by reducing the yield of the reaction (**Scheme 47**). *N,N'*-Dicyclohexylurea (DCU) was formed as a by-product.

It is known[75] that reaction conditions that favour intermolecular nucleophilic attack on the O-acylisourea (**77**) lead to fewer side reactions, and to cleaner products, because N-acylurea (**79**) formation is suppressed. For example, N-acylurea formation is less likely to occur when reactions are run in solvents with low dielectric constants (CCl_4, CH_2Cl_2, C_6H_6), than in high dielectric constant solvents (DMF, acetonitrile, DMSO). N-acylurea formation can also be avoided, or reduced dramatically, by performing the coupling reaction in the presence of a nucleophile which will react very

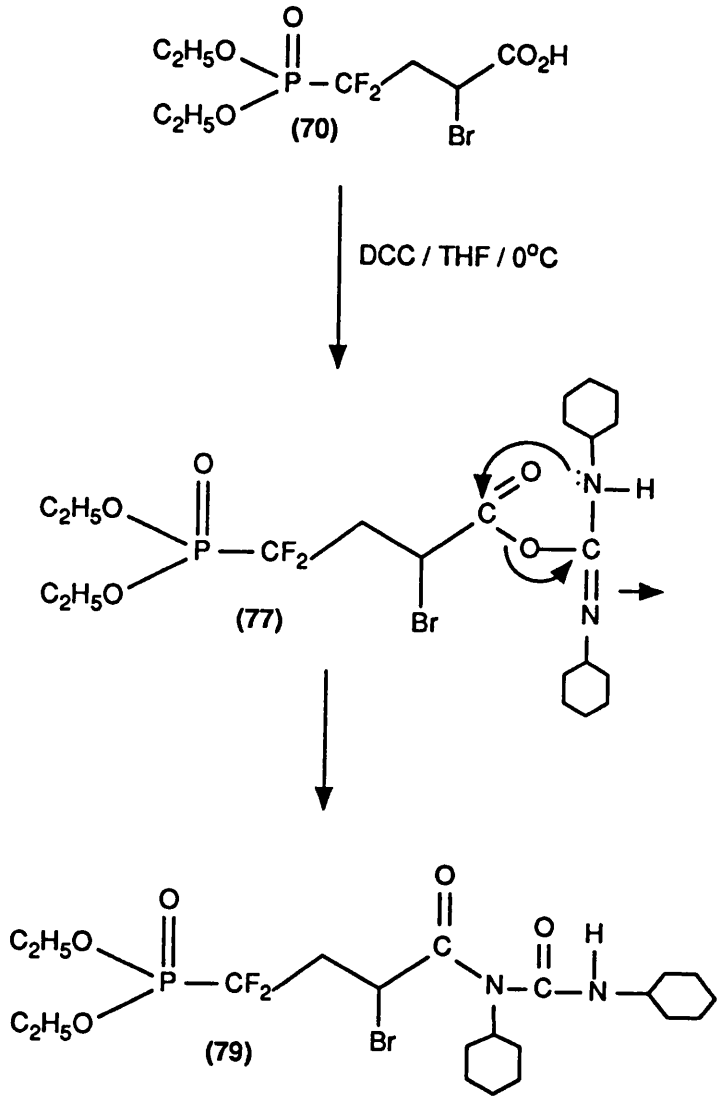
rapidly with the O-acylisourea to give an acylating agent, which is still reactive enough for aminolysis, but which is more discriminating and does not lead to racemization or other side reactions.



Scheme 46

DMAP has been shown [76] to have high catalytic activity in acyl transfer reactions, in which it specifically facilitates the acylation of sterically hindered and other deactivated alcohols. B. Neises *et al.* found that the addition of 3-10 mol% DMAP accelerates the DCC-activated esterification of carboxylic acids with alcohols or thiols to such an extent that formation of side products is suppressed, and even sterically demanding esters are formed in good yields at room temperature.

Screen *et al.*[77] proposed the following mechanism for the acylation of alcohols using DCC and DMAP as catalyst (**Figure 27**):


O
C2H5O
P – CF2
CO2H
C2H5O
Br
(70)
DCC / THF / 0°C
O
C2H5O
P — CF2
C2H5O
(77)
Br
O
C
O
N — H
O — C
N
O
C2H5O
P — CF2
C2H5O
(79)
Br
O
C
O
H
N — C — N

Scheme 47

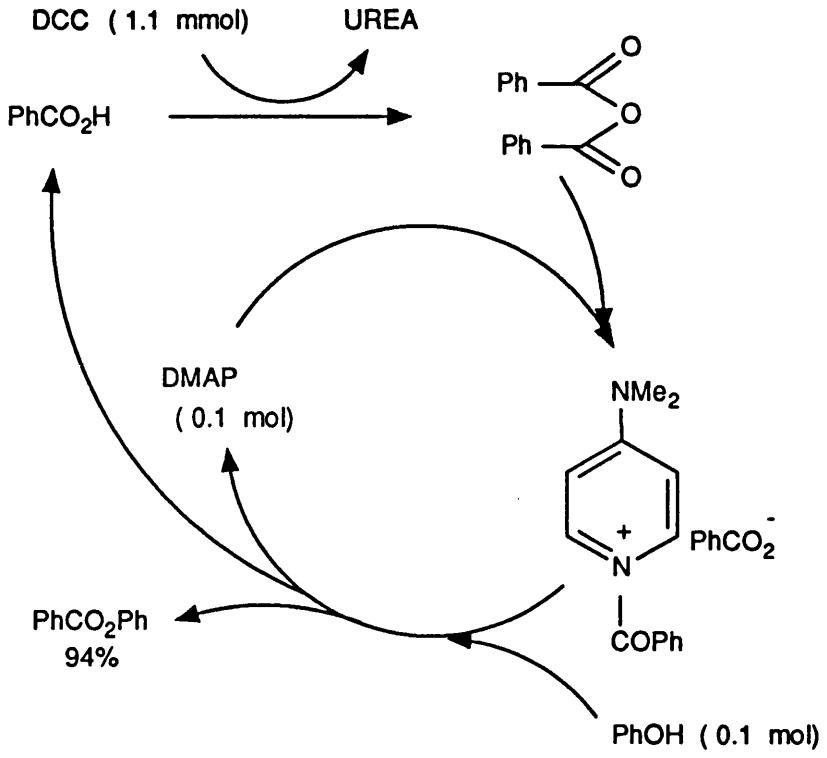


Figure 27 - Proposed mechanism for the acylation of alcohols

Given the reasons described above, the compound (**70**) was reacted with [(1S)-endo]-(-)-borneol using DMAP as catalyst in DCM at 0°C. The reaction was completed in 4 hours with the formation of compound (**78**) in 95% yield. There was no formation of N-acylurea (**79**) as a by-product.

Analysis of compound (**78**) and (**79**) indicated the elemental composition of $C_{18}H_{30}BrF_2O_5P$ and $C_{21}H_{36}BrF_2O_5P$, which was in agreement with the mass spectrum (MH^+ 475,447 18% and MH^+ 545,547 10% respectively).

Analysis of the ^1H NMR spectrum of compound (**78**) was possible by comparison of the ^1H NMR of borneol and compound (**70**). Although it was possible to assign all the protons resonances, measurement of the all coupling constants was difficult due to the complex multiplets for each proton.

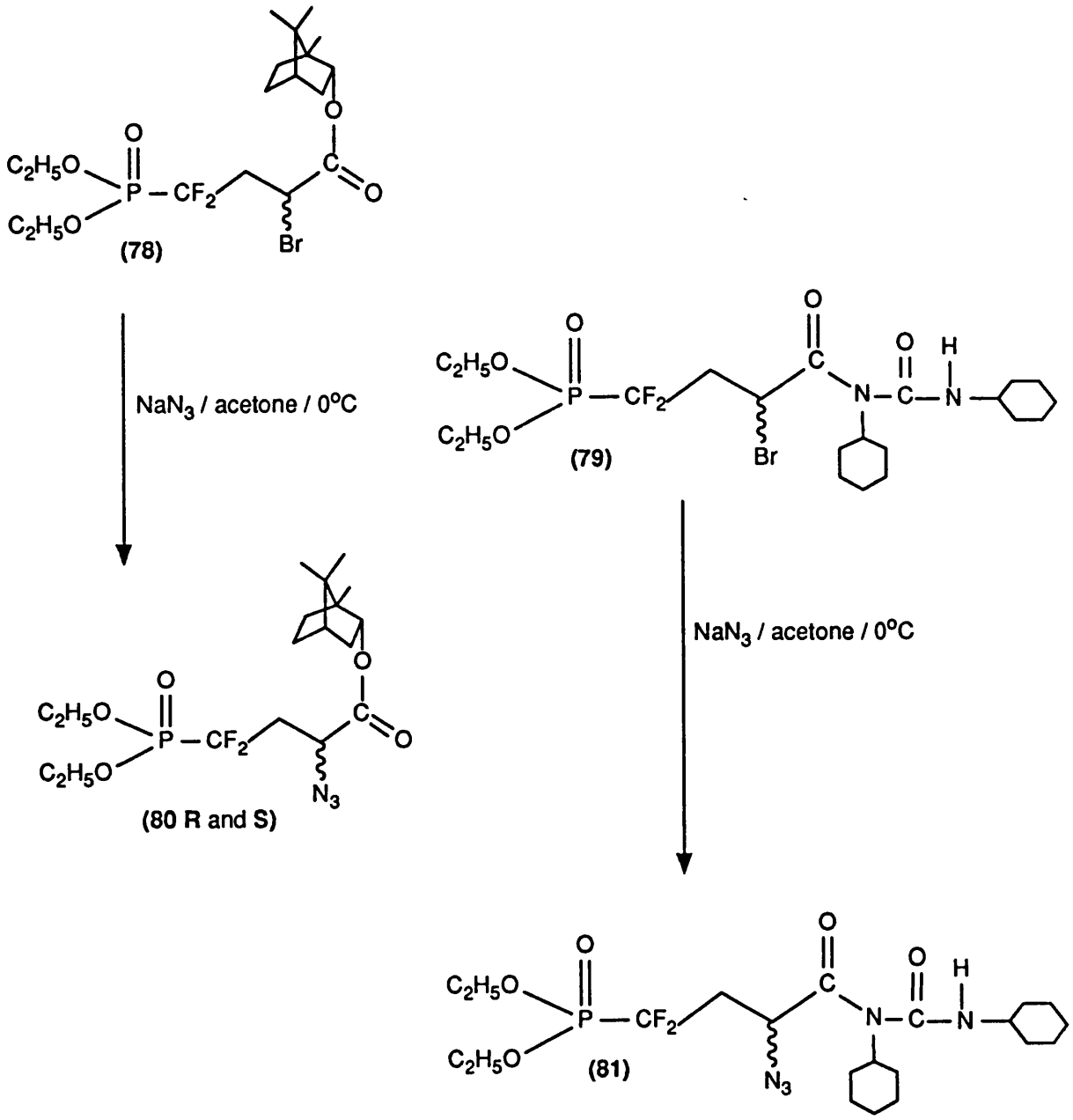
Analysis of the ^{1}H NMR and ^{13}C NMR spectra of compound (**79**) was also possible by comparison of ^{1}H NMR, ^{13}C NMR spectra of DCC, and compound (**70**). The presence of two carbonyl groups in compound (**79**) was evident from the IR spectrum, which contained two distinct carbonyl bands at 1760 and 1660 cm^{-1}, and also from the ^{13}C NMR spectrum which showed two singlets at δ 152.7 and 165.0 ppm.

2.7.6 Formation of Azides from Compound (78) and (79)

The reaction of compound (**78**) and (**79**) with sodium azide in acetone/water (7:3) at 30°C proceeded successfully to give the corresponding azide compounds (**80**) and (**81**) (**Scheme 48**), which were separated using column chromatography.

The compound (**80**) was isolated as a pale oil in 65% yield, and the compound (**81**) was isolated as a white solid in 66% yield. Recrystallization of compound (**81**) from ethylacetate/petrol ether gave crystals of suitable quality for a successful X-ray crystallographic determination (**Figure 28**); full details are in **Appendix 2.**

Analysis of compound (**80**) and (**81**) indicated the elemental composition of $C_{18}H_{30}F_2N_3O_5P$ and $C_{21}H_{36}F_2N_5O_5P$, which was in agreement with the mass spectrum (MH^+ 438, 18% and MH^+ 508, 8% respectively). The IR indicated the presence of the azido group (ν_{max} 2110 cm^{-1} for compound (**80**), and 2107 cm^{-1} for compound (**81**)).



Scheme 48

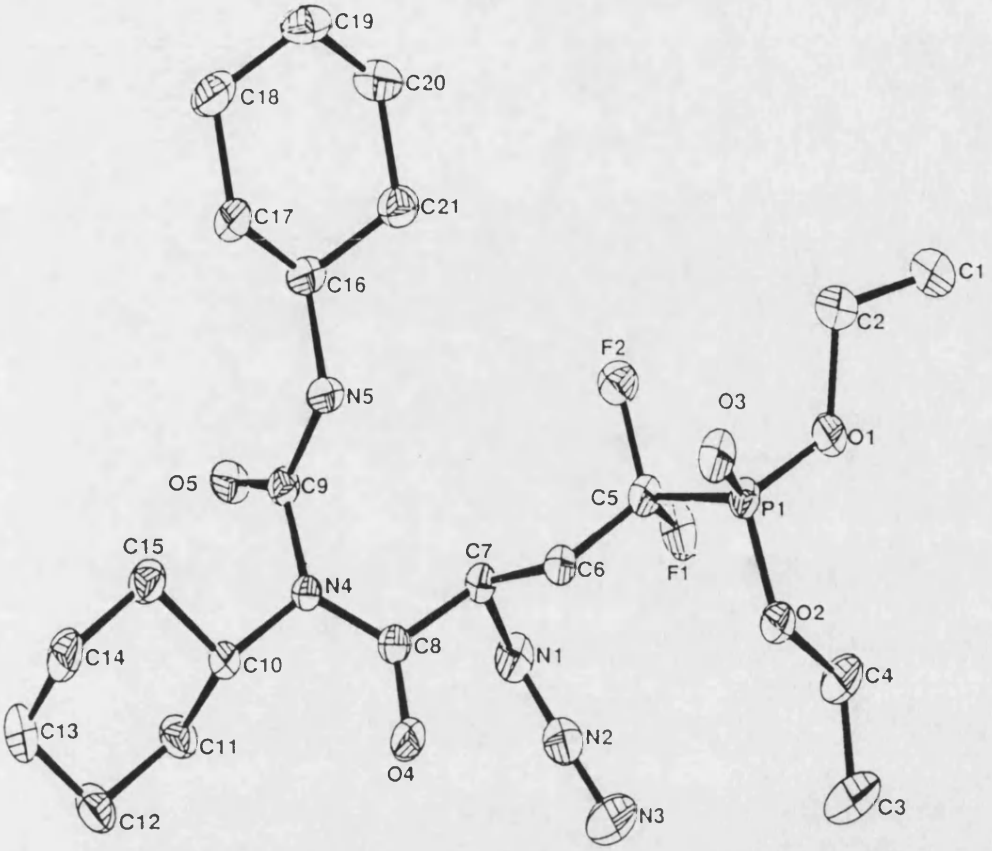


Figure 28 - ORTEP diagram of compound **(81)**

The ^{1}H NMR spectrum of compounds (**80**) and (**81**) was similar to the previous compounds (**78**) and (**79**), which facilitated the analysis and assignments of the protons of compounds (**80**) and (**81**). The signal for the 2-H protons moved upfield to 4.30 ppm for compound (**80**) and to 4.27 ppm for compound (**81**), where they overlapped with the signal from the methylene protons of the ethyl groups. The ^{19}F NMR and ^{31}P NMR (coupled and decoupled) spectra of (**80**) also helped in the assignments of the protons

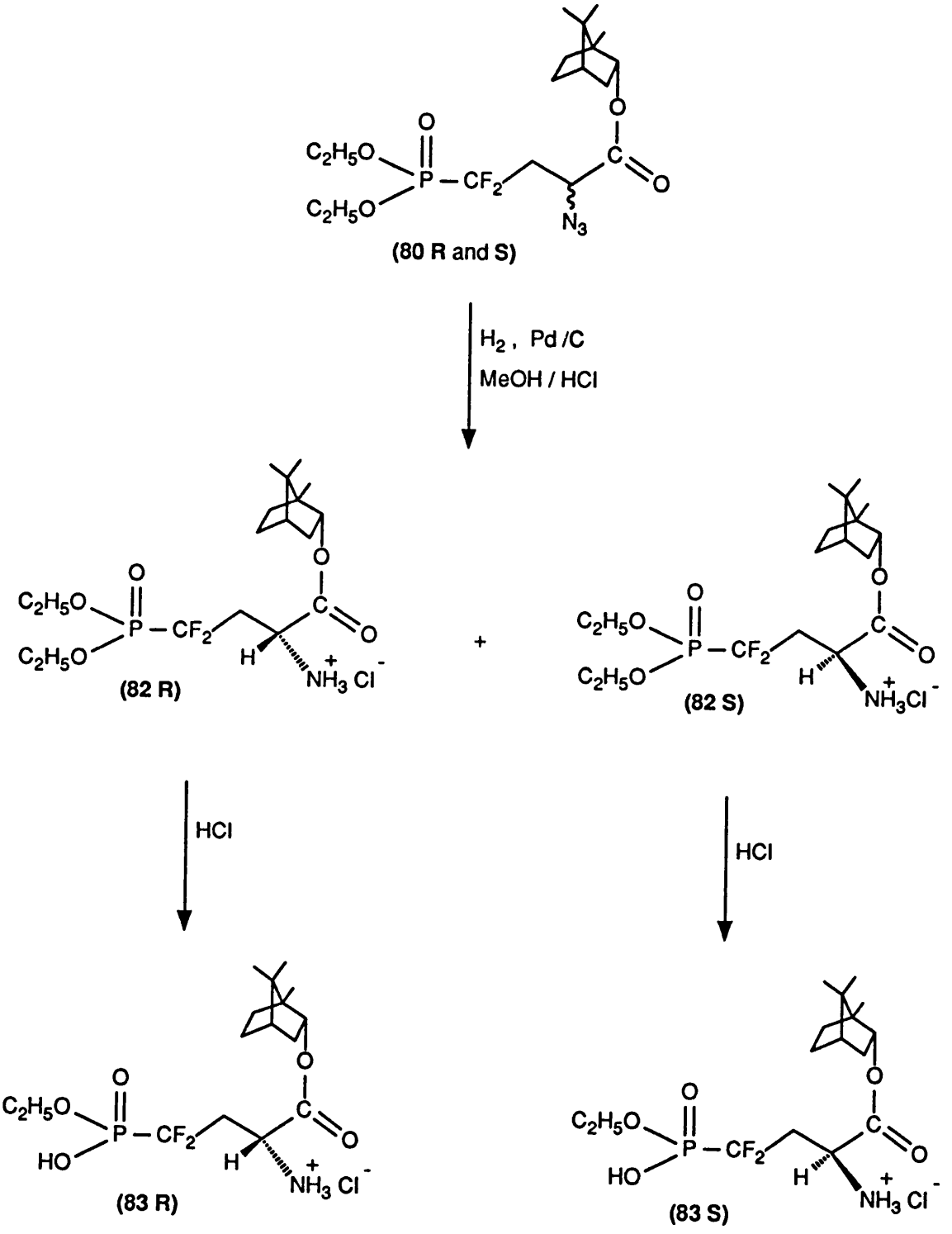
3a, 3b and 2-H. The ^{19}F NMR spectrum of (**80**) was a typical AB pattern since the two fluorine atoms are non-equivalent. The coupling constant between the two fluorine atoms was 300.5 Hz and the fluorine-phosphorus coupling constant is 104.2 Hz. The coupling constants of the two vicinal hydrogens with fluorine atoms were observed at 25.0 Hz and 19.7 Hz. The coupling constant of the 2-H proton with fluorine was 12.7 Hz. The phosphorus-fluorine coupling constant, $J_{P,F}$= 105 Hz, was observed in the ^{31}P NMR (decoupled), appearing as a triplet. The ^{31}P NMR spectrum (coupled) allowed the $J_{P,3a}$= $J_{P,3b}$= 6.9 Hz to be measured.

The presence of two carbonyl groups in compound (**81**) was evident from the IR spectrum which contains two distinct carbonyl bands at 1706 and 1660 cm^{-1}, and also from the ^{13}C NMR spectrum which showed two signals at δ 152.5 and 165.7 ppm.

2.7.7 Hydrogenation of Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butyrate (80)

Reduction of bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butyrate (**80**) by catalytic hydrogenation over 5% Pd/C using methanol as solvent in the presence of $HCl_{(conc.)}$, afforded a mixture of bornyl [4,4-difluoro-4-(diethoxy phosphinyl)-2-amino] butyrate hydrochloride salt, (**82 R** and **82 S**), and bornyl [4,4-difluoro-4-(ethoxyhydroxyphosphinyl)-2-amino] butyrate hydrochloride salt, (**83 R** and **83 S**)(**Scheme 49**), which were separated by column chromatography.

The amine salts (**82 R**) and (**82 S**) were a 53:47 mixture of diastereoisomers (by mass) obtained in 50.6% yield. Compounds (**83 R**) and (**83 S**), a 85:15 mixture of diastereoisomers (by mass), obtained in 14.6% yield, are thought to result from hydrolysis of one of the ethyl ester groups of the compounds (**82 R**) and (**82 S**) by the hydrochloric acid present in the reaction mixture.



Scheme 49

The structure of the diastereoisomers (**82 R**) and (**82 S**) was determined by comparison of the ^{1}H NMR and ^{13}C NMR spectra with the previous compound (**80**). The R and S configurations could be distinguished by building a molecular model for the diastereoisomers (**82 R** and **82 S**). The long-range coupling between the 2-H and F was observed when they adopted the"W conformation"[112]. The molecular model indicated that only in the diastereoisomer (**82 R**) could the 2-H and F adopt the "W conformation". Therefore, in the ^{1}H NMR, the signal of the 2-H proton appeared as ddd ($J_{2,3a}$ = 4.87 Hz, $J_{2,3b}$ = 7.63 Hz, $J_{2,F}$ = 3.35 Hz) resulting from the coupling with 3-H proton and fluorine. In the diastereoisomer (**82 S**) the signal of the 2-H proton appeared as a doublet of doublets due to the coupling of the 2-H proton with 3-H proton only ($J_{2,3a}$ = 4.58 Hz, $J_{2,3b}$ = 7.69 Hz).

The signal of the 2-H proton moved upfield to 3.97 ppm in the S diastereoisomer and to 3.73 ppm in the R diastereoisomer. As was expected, the ^{1}H NMR and ^{13}C NMR spectra of the two diastereoisomers (**82 R**) and (**82 S**) were similar. The TLC of the mixture of (**82 R**) and (**82 S**) showed two spots close together, with Rfs of 0.75 and 0.65 ($CHCl_3$/MeOH 90:10).

The elemental composition $C_{18}H_{32}F_2NO_5P.HCl$ for both diastereoisomers (**82 R**) and (**82 S**) was indicated by mass spectrum m/z(+ve FAB) 412(MH^+ 100%).

The compounds (**83 R**) and (**83 S**), which are more polar than compounds (**82 R**) and (**82 S**) due to the presence of the OH group, and which resulted from hydrolysis of the ethyl ester group, also showed two spots close together with Rfs of 0.28 and 0.18 ($CHCl_3$/MeOH 90:10).

The ^{1}H NMR and ^{13}C NMR spectra of (**83 R**) and (**83 S**) were similar to those of compounds (**82 R**) and (**82 S**). The signal of the 2-H proton moved downfield to 4.44 ppm in the S diastereoisomer and to 3.99 ppm in the R diastereoisomer, due to the presence of the OH, which is more polar than the ethyl ester group.

The elemental composition $C_{16}H_{31}F_2NO_5P.HCl$ for both diastereoisomer (**83 R**)

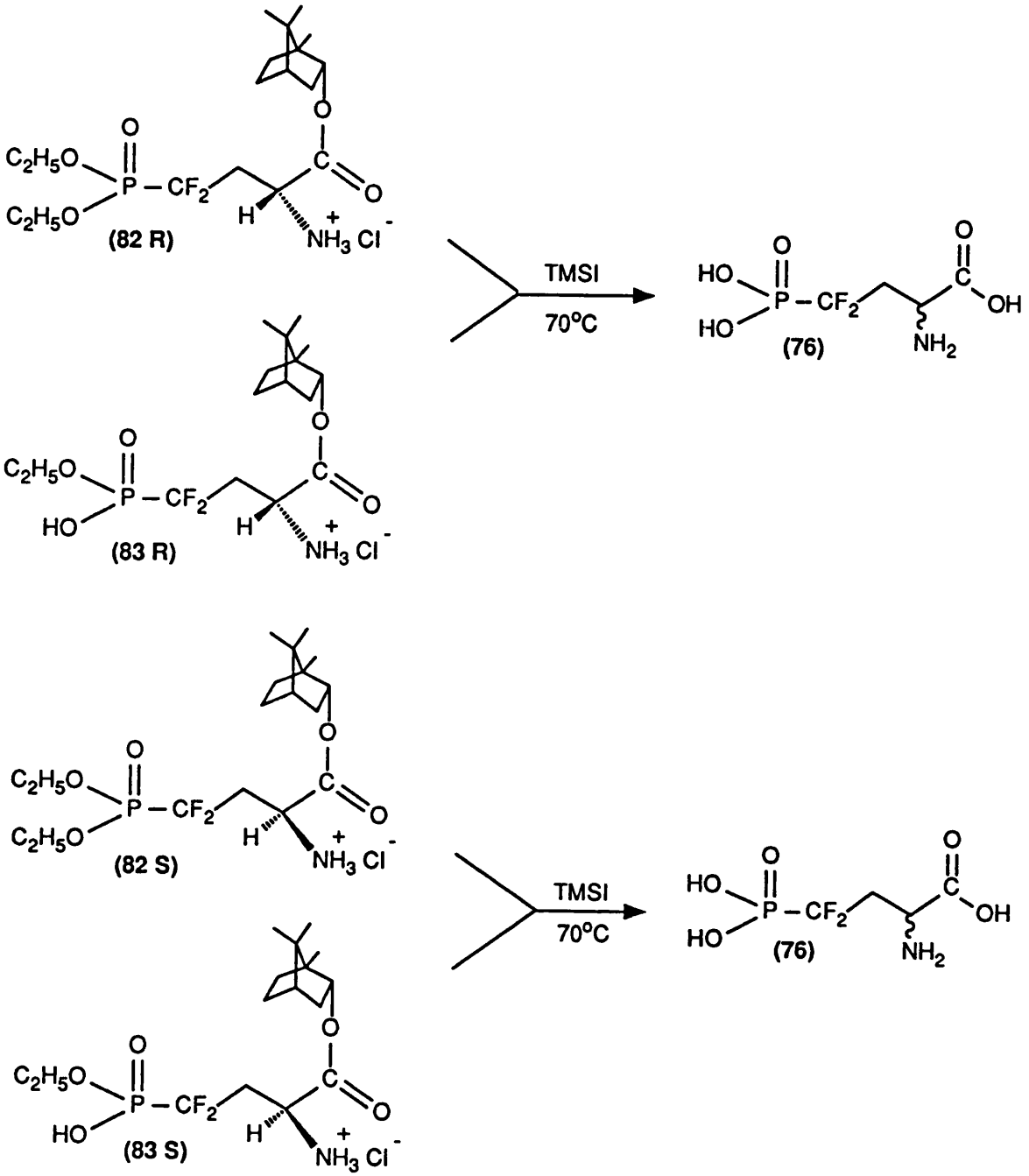
and (**83 S**) was confirmed by mass spectrum m/z(-ve FAB) 382(MH^- 100%).

2.7.8 Deprotection of (Diethoxyphosphinyl) and Bornyl ester of compounds (82 R), (82 S), (83 R) and (83 S)

The final stage in the synthesis of our target compound, (R,S)[4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**76**), was the conversion of the ethoxyphosphinyl and the bornyl ester to hydroxyl groups, and the ammonium salt to free amine (**Scheme 50**).

Reaction of bornyl (4,4-difluoro-4-(ethylhydroxyphosphinyl)-2-amino butyrate hydrochloride salt (**83 S**), with TMSI at r.t. in $CHCl_3$, followed by hydrolysis in water, gave only the deprotection of the diethoxyphosphinyl group. Deprotection of the bornyl ester was achieved by reaction with NaOH (2N), which afforded the desired compound, (S)[4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**76 S**), an analogue of L-phosphoserine, as a white solid in 90%, yield. The elemental composition $C_4H_8F_2NO_5P$ of compound (**76**) was indicated by mass spectrum, m/z(+ve FAB) 220(MH^+, 12%). The strong band in the IR at 3425 cm^{-1} indicated the presence of the amine group.

In the ^1H NMR spectrum of compound (**76**), the resonance due to the 2-H proton appeared as a doublet of doublets centered at 4.5 ppm, resulting from the coupling with the 3-H proton ($J_{2,3a}$= 3.4Hz, $J_{2,3b}$= 8.9Hz). The 3-H proton appeared as a multiplet centered at 2.9 ppm. The ^{19}F NMR spectrum of compound (**76**) appeared as an AB pattern since the fluorine atoms are diastereotopic. The coupling constant between the two fluorines atoms was 290.2Hz, with a fluorine-phosphorus coupling constant of 91.3Hz. The coupling constants of the vicinal hydrogens with fluorines of 23.1Hz and 15.1Hz were observed.



Scheme 50

The product (**76 S**), as expected, was optically pure, $[\alpha]_D$= 30.4° (c 4.60, H_2O). However, racemization of compound (**76**) occurred upon standing in aqueous solution for one week ($[\alpha]_D$= 0.0). Since the resulting compound, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**76**), is a strong acid, it could lead to autoracemization. There were no changes in the ^1H NMR and ^{13}C NMR spectra.

According to the literature, the fully protected (α,α)-difluoro alkyl phosphonate, an analogue of L-phosphoserine, which was synthesized by Berkowitz *et al.*[24], showed $[\alpha]_D$= -10.8° (c 2.20, MeOH).

Deprotection of compound (**83 S**) under different conditions, using TMSI without solvent at 70°C followed by hydrolysis in water, resulted in the racemization of compound (**76**) during the reaction.

Reaction of bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloride salt (**82 S**) with TMSI without solvent at 70°C followed by hydrolysis in water, gave complete deprotection of the diethoxyphosphinyl group and bornyl ester, and also resulted in the racemization of the expected amino acid, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**76**), during the reaction. The expected (S) stereochemistry was not observed.

Deprotection of the diethoxyphosphinyl group and the bornyl ester of bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloride salt (**82 R**) and bornyl (4,4-difluoro-4-(ethylhydroxyphosphinyl)-2-amino butyrate hydrochloride salt (**83 R**) was carried out by reacting compounds (**82 R**) and (**83 R**) with TMSI without solvent at 70°C, followed by hydrolysis in water. The expected (R) stereochemistry for the resulting compound, 4,4-difluoro-4-(dihydroxy phosphinyl)-2-amino] butanoic acid (**76**), was not observed; racemization of compound (**76**) occurred during the reaction with TMSI.

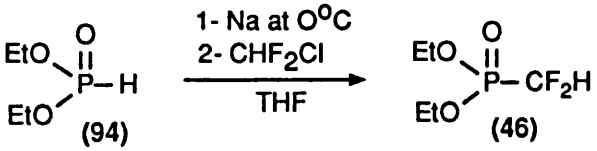
The ^1H NMR and ^{13}C NMR spectra of the racemic compound (**76**), resulting from deprotection of the compound (**82 R**) and (**83 R**) were the same to the compound (**76**) resulting from the deprotection of compounds (**82 S**) and (**83 S**).

The elemental composition $C_4H_8F_2NO_5P$ was confirmed by mass spectrum m/z(ve +FAB) 220(MH^+, 12%)

2.8 Synthesis of Methyl [3,3-difluoro-3-(diethoxyphosphinyl) -2-hydroxy-2-methyl] propionate (84)

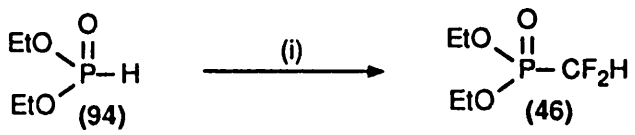
2.8.1 Preparation of Diethyl(difluoromethyl) phosphonate (46)

The synthesis of the diethyl (difluoromethy) phosphonate was reported by Bergstrom *et al* [42]. The reaction was carried out by the treatment of diethyl phosphite (**94**) with sodium metal, and then with chlorodifluoromethane to give diethyl (difluoromethyl) phosphonate (**46**) in 54% yield (**Scheme 51**).



Scheme 51

In this project, the synthesis of the diethyl (difluoromethyl) phosphonate was prepared by reacting diethyl phosphite with sodium hydride 80% in dry ether at 0°C under N_2, followed by addition of chlorodifluoromethane at 0°C, to give a colourless oil in 75.6% (**Scheme 52**).



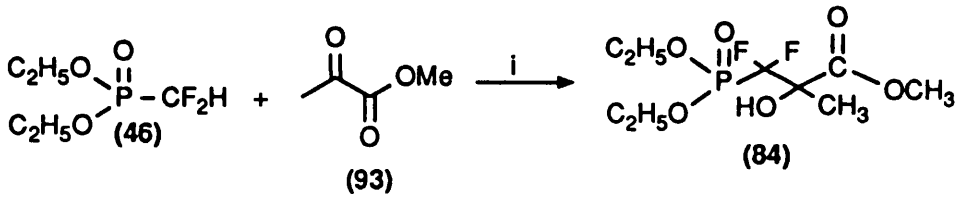
Scheme 52 - Reagents and conditions: (i) ether (dry), NaH, CHF_2Cl, 0°C, 4h.

By changing the solvent from ether to THF, it was possible to increase the yield to 95%. This can be explained by the fact that this reaction involves substitution by an S_N2 mechanism, and the rate of S_N2 reactions are affected by the solvent. Polar aprotic solvents, which have strong dipoles but do not have -OH or -NH groups, are the best and, since THF (dipole moment = 1.63 debyes) is more polar than ether (dipole moment = 1.15 debyes), it was used to improve the yield of the reaction.

The ^1H NMR spectrum showed a well defined doublet of doublets of doublets at 5.97 ppm resulting from the resonance of methine proton with fluorine and phosphorus ($J_{H,F}$ = 48.7 Hz and $J_{H,P}$ = 26.9 Hz).

2.8.2 Coupling Reaction of Diethyl (difluoromethyl)phosphonate with Methyl pyruvate

Methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-hydroxy-2-methyl] propionate (**84**) was prepared by treatment of diethyl (difluoromethyl)phosphonate (**46**) with lithium diisopropylamine (LDA) in dry tetrahydrofuran (THF) at -78°C, followed by the addition of methyl pyruvate (**93**) (**Scheme 53**).



Scheme 53 - Reagents and conditions: (i) THF(dry), BuLi, Diisopropylamine, -78°C, 7h, (30%).

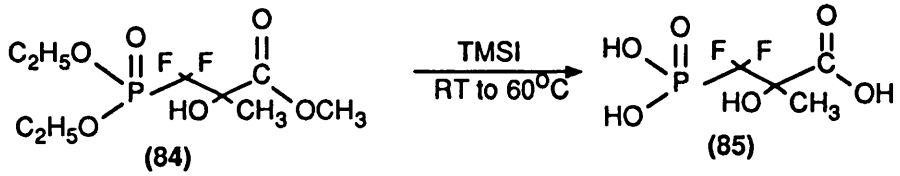
The product was extracted with ether and dried over Na_2SO_4. It was purified by column chromatography to give an amber oil in 43% yield.

The proton NMR spectrum of compound (**84**) shows two singlets at 1.6 ppm and 3.9 ppm assigned to the CH_3 and OCH_3 respectively. The ^{19}F NMR spectrum is a typical AB pattern since the two fluorine atoms are diastereotopic. The coupling constant between the two fluorine atoms is 306.9 Hz, with a fluorine-phosphorus coupling constant of 102.3 Hz. The ^{31}P NMR (decoupled) showed a triplet centered at 5.1 ppm, resulting from the coupling of phosphorus with the two fluorine atoms ($J_{P,F}$ = 101.1 Hz).

Analysis of compound (**84**) indicated the elemental composition of $C_9H_{17}F_2O_6P$, which was in agreement with the mass spectrum (MH^+ 291, 100%).

2.9 Synthesis of [3,3-Difluoro-3-(dihydroxyphosphinyl)-2-hydroxy -2-methyl] propionic acid (85)

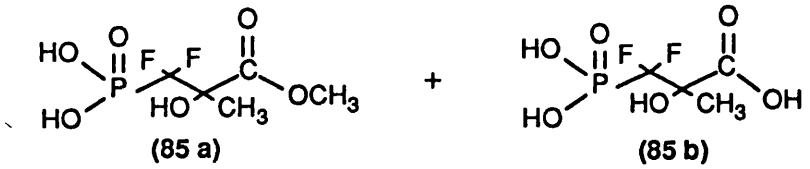
[3,3-Difluoro-3-(dihydroxyphosphinyl)-2-hydroxy-2-methyl] propionic acid (**85**) was prepared by hydrolysis of methyl [3,3-difluoro-3-(diethoxyphosphinyl) -2-hydroxy-2-methyl] propionate (**84**) using iodotrimethylsilane. The reaction was carried out at 60°C in the absence of solvent and required 7 days to complete (**Scheme 54**).



(84) (85)

Scheme 54

The product was extracted with water and freeze dried giving a dark brown viscous liquid. Initially the reaction was carried out using solvent (THF, ether, DCM), and it was verified that the reaction was not complete even after 3 weeks, yielding a mixture of the two compounds (**85 a** and **85 b**) shown below.



(85 a) + (85 b)

The ^1H NMR analysis in D_2O showed just one singlet due to CH_3 at 1.42 ppm. The elemental composition $C_4H_7F_2O_6P$ was indicated by the mass spectrum and confirmed by accurate mass measurement (-ve FAB 218.9861, 100%).

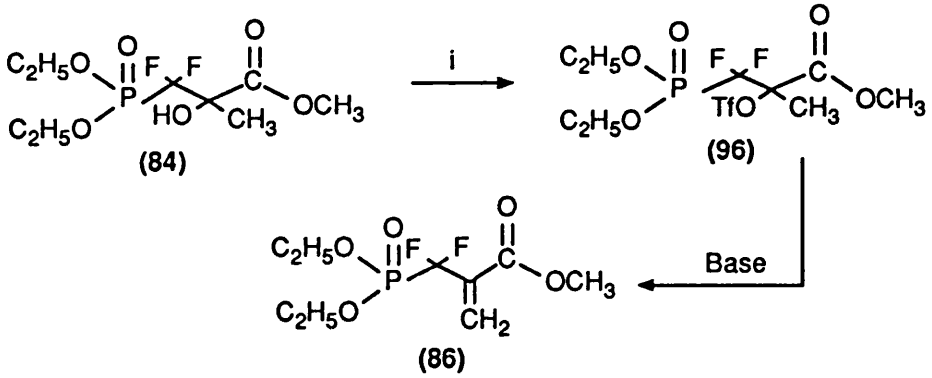
2.10 Attempted preparation of Methyl 2-[(diethoxyphosphinyl) difluoromethyl] propenoate (86)

Another important reaction considered in this project was the dehydration of methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-hydroxy-2-methyl] propionate (**84**) to give the corresponding olefin, methyl 2-[(diethoxyphosphinyl) difluoromethyl] propenoate (**86**), which is an analogue of PEP and could be an potential inhibitor in the shikimic acid pathway.

Several attempts were made to synthesise the product. Firstly, the method of Hoffman *et al.*[78] for dehydration of a secondary alcohol was investigated. The alcohol (84) and the catalyst $CuSO_4$ were heated from 100 - 200°C in a small kugelrohr distillation apparatus for 4 hours, but the NMR spectrum showed only starting material. Secondly, we tried dissolving the alcohol (84) and the catalyst $CuSO_4$ in dry toluene and refluxing at 120°C for 6 hours. The reaction was followed by TLC, but the alcohol (84) decomposed instead of giving the desired product.

Another attempt involved reflux with iodine, but this was again unsuccessful.

Since these attempts were unsucessful other methods were tried. Conversion of the hydroxyl group to a triflate or acetate, followed by elimination was investigated (Scheme 55).



Scheme 55 -Reagents and Conditions : (i) Tf_2O, Py, CH_2Cl_2(dry), -23°C to RT, 3 days reaction

The reaction of the alcohol with trifluoromethane sulfonic anhydride (triflate) was successful, but the elimination from (96) was not successful. LDA was used as the base for the elimination reaction, but (96) decomposed into four or five other products, and did not give the desired olefin. Other bases, such as pyridine, DBU, Et_3N, and potassium *tert*-butoxide, were also used, but they did not give the desired compound.

Posner *et al*[79] used Woelm alumina at room temperature for high-yield

dehydrosulfonation of some secondary cyclic and acyclic alcohols, and some primary sulfonate esters. An attempt to synthetize the compound (**86**) using basic alumina (Brockman I) for the elimination reaction was made, but only starting material was observed after several days.

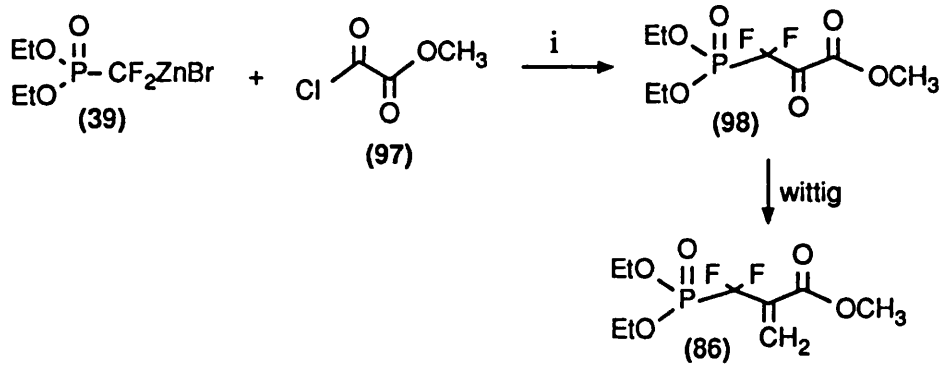
Other attempts to dehydrate the compound (**84**) to give the corresponding olefin (**86**) are listed below, but were unsuccessful.

- Dehydration by using DAST, following the method described by Blackburn *et al.*[64].

- Reaction of the OH group of the compound (**84**) with acetyl chloride and mesylchloride, and then elimination with a strong base (LDA, NAH or DBU).

- Dehydration of the compound (**84**) using the Martin sulfurane dehydrating agent, bis[α,α-bis(trifluoromethyl)benzenemethanolato]diphenylsulfur. There was no reaction.

Therefore, another strategy to obtain the desired olefin was tried. The Wittig reaction of methyl 2-oxo-3,3-difuoro-3-(diethoxyphosphinyl) propionate (**98**), prepared by reacting [(diethoxyphosphinyl) difluoromethyl] zinc bromide (**39**) with methyl oxalyl chloride (**97**) in the presence of catalytic amount of cuprous bromide at r.t. in THF, **Scheme 56**, was expected to give the compound (**86**).



Scheme 56 - Reagents and conditions: (i) CuBr, R.T., 20-24 h, monoglyme(dry), 62%

The first attempted Wittig reaction followed the method described by Takai *et al.*[82]. Methyl 2-oxo-3,3-difluoro-3-(diethoxyphosphinyl) propionate (**98**) was reacted with a mixture of CH_2I_2, Zn, and trimethyl aluminium (1:3:0.2 mol ratio) but, unfortunately, the compound (**98**) decomposed and the desired compound (**86**) was not formed.

The second attempt was the Wittig reaction using the method described by Lyly *et al.*[83]. Methyl 2-oxo-3,3-difluoro-3-(diethoxyphosphinyl) propionate (**98**) was reacted with methyl triphenylphosphonium bromide and butyl lithium in dry THF at -78°C, but unfortunately this method did not work either.

The third attempt was the Wittig reaction using Tebbe Reagent (μ-chloro-μ-methylene bis (cyclopentadienyl) titanium dimethylaluminium), according to the method described by Phillion *et al.*[56]. Unfortunately, this method did not give the desired olefin.

CHAPTER THREE

EXPERIMENTAL

CHAPTER THREE - EXPERIMENTAL

3.1 Instrumentation and Experimental Techniques

3.1.1 General

Glassware used for moisture sensitive reactions was heated in an oven at 120°C for approximately 12 hours, and then allowed to cool in a desiccator over $CaCl_2$. Flasks and stirrer bars were additionally flame-dried under a stream of dry nitrogen prior to use.

Solvents were evaporated with a Büchi rotary evaporator using a water aspirator or a vacuum pump as required, and a water bath at room temperature to avoid unnecessary heating. All yields quoted are of purified products, and are uncorrected.

3.1.2 Analysis and Spectroscopy

Melting points (m.p.) were determined on commercially available apparatus (Electrothermal melting point apparatus), or Büchi 510, and are uncorrected. Elemental microanalysis were carried out using a Carlo Erba 1106 Elemental Analyser. Optical rotations were measured using a Perkin-Elmer 141 polarimeter with concentration (*c*) expressed in g/100 cm^3.

Infrared spectra were recorded in the range of 4000-600 cm^{-1}, using a Perkin Elmer 1600 FT-IR spectrophotometer and peaks are reported (ν_{max}) in wave numbers (cm^{-1}). Spectra of liquid samples were taken as thin films on sodium chloride plates. Spectra of solid samples were taken as nujol mulls, or in chloroform solution, as indicated.

Proton NMR (^1H nmr) spectra were recorded on a Jeol GX FT-270 (270 MHz) spectrometer although, where indicated, a Jeol GX FT-400 (400 MHz) spectrometer

was used. Carbon 13 magnetic resonance (^{13}C nmr) spectra were recorded on a Jeol GX FT-270 spectrometer operating at 67.8 MHz and using 90 and 135 DEPT pulse sequences to aid multiplicity determination. Chemical shifts (δ) are expressed in parts per million downfield from internal tetramethylsilane (TMS). The multiplicities of the resonances are denoted by: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The abbreviation "br" (broadened) is used to indicate significant broadening, whether due to rapid exchange or unresolved fine coupling. Nuclear Overhansen effect, 2D homonuclear shift correlated (COSY) spectra were used to confirm proton assignments when required.

Mass spectra were recorded using a VG Analytical 7070 E instrument with a VG 2000 data system. Electron ionisation (E.I.) was produced using an ionising potential of 70 eV. Chemical ionisation (C.I.) was employed using isobutane as the reagent gas although, where indicated, ammonia was also used.

3.1.3 Solvents and Reagents

All general reagents and solvents were purified and dried when required, using the methods described in D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, "Purification of Laboratory Chemicals", Pergamon Press, Oxford, 1980. Petrol refers to petroleum ether boiling point in the range of 60-80°C, and light petrol refers to that boiling point in the range of 40-60°C. Tetrahydrofuran (THF) was pre-dried over sodium wire, and then refluxed over sodium benzophenone ketyl under nitrogen atmosphere until anhydrous. This was redistilled immediately prior to use.

3.1.4 Chromatography

Thin layer chromatography (TLC) was used extensively as a qualitative guide during reactions, and for assessing the purity of compounds. Merck DC-alufolien

kieselgel 60 F_{254} sheets containing fluorescent indicator were used for this purpose. Visualisation of compounds was achieved by illumination under short wavelength (254nm) ultraviolet light (when possible), and developing with ammonium molybdate/sulfuric acid/water solution, followed by warming of the T.L.C. plate.

Medium pressure flash column chromatography was routinely employed using Amicon Matrex silica gel for reaction component separations. Columns were packed as a slurry in the eluting solvent, and material to be chromatographed introduced directly as a solution in the eluting solvent, or pre-absorbed onto silica gel and then applied as thin layer to the top of the column. The eluting solvent was employed as a gradient and the pressure was developed using a small hand bellow (GallenKamp).

3.2 Experimental Procedure

Methyl 2-[2',2'-difluoroethyl-2'-(diethoxyphosphinyl)] propenoate (67)

To the solution of [(diethoxyphosphinyl) difluoromethyl] zinc bromide (5.41mmol; 1.80g) in 6ml of dry THF was added a catalytic amount of cuprous bromide (0.1g). Then, methyl 2- (bromethyl) acrylate was added dropwise at room temperature, and the mixture was stirred overnight. The mixture was filtered and poured into 10ml of H_2O and extrated with ether (3x10ml). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified using column chromatography on silica gel with ethyl acetate - light petroleum (b.p. 60-80°C) (3:7) as the eluent to give the product in 48.8% yield; R_f= 0.48(petrol-ethyl acetate 1:1); υ_{max}(liquid film)/cm^{-1} 3502, 2988, 1725(C=0), 1634(C=CH_2), 1441, 1395, 1311, 1274(P=O), 1196, 1161, 1096, 1042(OCH_3), 1017, 896; δ_H($CDCl_3$) 1.39(t, $C\underline{H}_3CH_2OP$, J= 7.05Hz), 3.09-3.25(td, $CF_2C\underline{H}_2$, $J_{H,F}$= 19.64Hz, $J_{H,P}$= 4.76Hz), 3.79(s, OCH_3), 4.29(m, $CH_3C\underline{H}_2OP$), 5.89(s, vinylic hydrogen), 6.47(s, vinylic hydrogen); δ_C($CDCl_3$) 16.2(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5.5Hz), 34.89(td, $CF_2\underline{C}H_2$, $J_{C,F}$= 21.15Hz, $J_{C,P}$=

16.53Hz), 52.02 (s, OCH_3), 64.42(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 7.3Hz), 118.79(td, CF_2, $J_{C,F}$= 261.5Hz, $J_{C,P}$= 216.7Hz), 131.15(s, C=$\underline{C}H_2$), 166.45(s, C=O); m/z(E.I.) 286(M^+, 34%), 255(M^+ - OMe, 25), 199(58), 109(100); m/z(C.I.) 287(MH^+, 100%). Anal. Calcd. for $C_{10}H_{17}F_2O_5P$: C, 42.0; H, 6.0; Found: C, 42.1; H, 6.10.

Alternative method by Radical Reaction

To a solution of tributyltin methyl acrylate (1.28mmol; 0.5g) in dry toluene (5ml) was added diethyl difluoromethyl phosphonate (0.64mmol; 0.172g) in dry toluene (5ml). Then a catalytic amount of AIBN was added to the reaction mixture, and it was refluxed for 5h. Toluene was evaporated under reduced pressure. Column chromatography (petrol-ethyl acetate 7:3) yielded the title compound as a pale oil (18.4mg; 10%).

2-[2',2'-Difluoroethyl-2'-(diethoxyphosphinyl)] propenoic acid (68)

To the solution of [(diethoxyphosphinyl) difluoromethyl] zinc bromide (1.23g; 3.69mmol) in 6ml of THF(dry) was added a catalytic amount of cuprous bromide (0.2g). Then 2-bromo methyl acrylic acid (0.73g; 4.42mmol) was added, and the mixture was stirred overnight at room temperature. The mixture was filtered and poured into 10ml of H_2O and extracted with ether (3x10ml). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified using column chromatography on silica gel with chloroform/methanol (98.5:1.5) as the eluent, to give the product in 61% yield; R_f= 0.40($CHCl_3$-MeOH 9:1); ν_{max}(liquid film)/cm^{-1} 3498(COOH), 2987, 1725(C=O), 1634(C=CH_2), 1441, 1395, 1269(P=O), 1162, 1097, 1043; δ_H($CDCl_3$) 1.39(t, $C\underline{H}_3CH_2OP$, J= 7.08Hz), 3.17(td, $CF_2C\underline{H}_2$, $J_{H,F}$= 19.5Hz, $J_{H,P}$= 4.88Hz), 4.25-4.23(m, $CH_3C\underline{H}_2OP$), 5.98(s, vinylic hydrogen), 6.6(s, vinylic hydrogen); δ_C($CDCl_3$) 16.2(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5.5Hz), 34.6(td, $CF_2\underline{C}H_2$,

$J_{C,F}$= 29.2Hz, $J_{C,P}$= 16.5Hz), 64.8(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 7.7Hz), 118.1(td, CF_2, $J_{C,F}$= 261.1Hz, $J_{C,P}$= 217.1Hz), 133.0(s, C=$\underline{C}H_2$), 170.3(s, C=O); m/z(C.I.) 273(MH^+,100%); m/z(E.I.) 272(M^+, 2%), 227(M^+-CO_2H, 12), 201(M^+-$C(CH_2)CO_2H$, 13), 199(50), 109(100). Anal. Calcd for $C_9H_{15}F_2O_5P$: C, 39.7; H, 5.6; Found: C, 39.5; H, 5.6.

2-[2',2'-Difluoroethyl-2'-(dihydroxyphosphinyl)] propenoic acid (69) and 2-[2',2'-Difluoroethyl-2'-(ethoxyhydroxyphosphinyl propenoic acid (69a)

2-[2',2'-Difluoroethyl-2'-(diethoxyphosphinyl)] propenoic acid (**68**)(1.2g, 4.4mmol) in 10 ml of dry THF was stirred with TMSI(2.1g, 10.5mmol), under N_2 at r.t. for 6 h. The excess silylating reagent and ethyl iodide were removed URP to give the bis trimethyl silyl phosphonate esters, which were dissolved in ether (30ml) and then treated with water (20ml) to give a 1:1 mixture of two copmpounds: methyl [2',2'-difluoro-2'-(dihydroxy phosphonyl)] propenoic acid (**69**) and methyl [2',2'-difluoro-2'-(ethoxyhydroxy phosphonyl)] propenoic acid (**69a**) in 80% yield. These two compounds were separated by column chromatography ($CHCl_3$/MeOH 90:10 to 1:1).

The first band eluted (Rf= 0.62, chloroform-methanol 1:1) was collected, evaporated to afford methyl [2',2'-difluoro-2'-(ethylhydroxy phosphonyl)] propenoic acid (**69a**); ν_{max}(MeOD)/cm^{-1} 3853, 3743, 2927, 2361, 2071, 1698(C=O), 1650(C=C), 1496, 1049, 973; δ_H(MeOD) 0.44(t, $C\underline{H}_3CH_2OP$, J= 6.92Hz), 2.25(td, $CF_2C\underline{H}_2$, $J_{H,F}$= 19.7Hz, $J_{H,P}$= 4.11Hz), 3.3(m, $CH_3C\underline{H}_2OP$), 4.98(s, vinylic hydrogen), 5.5(s, vinylic hydrogen); δ_C($CDCl_3$) 16.8(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5.5Hz), 35.8(td, $CF_2\underline{C}H_2$, $J_{C,F}$= 32..2Hz, $J_{C,P}$= 20.2Hz), 65.2(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 5.5Hz), 120.7(td, CF_2, $J_{C,F}$= 260.1Hz, $J_{C,P}$= 213.6Hz), 131.6(s, C=$\underline{C}H_2$), 169.5(s, C=O); m/z(-ve FAB) 243(MH^- 100%), 221(14), 187(16), 79(40).

The second band eluted (Rf= 0.33, chloroform-methanol 1:1) was collected,

evaporated to yield methyl [2',2'-difluoro-2'-(dihydroxy phosphonyl)] propenoic acid (**69**); m.p. 72°C; $\nu_{max}(D_2O)/cm^{-1}$ 3424, 2527, 1700(C=O), 1630(C=C), 1438, 1332, 1209(P=O), 1109, 1053, 960; $\delta_H(D_2O)$ 3.14(dt, $CF_2C\underline{H}_2$, $J_{H,F}$= 20.4Hz, $J_{H,P}$= 2.47Hz), 5.89(s, vinylic hydrogen), 6.34(s, vinylic hydrogen); $\delta_C(D_2O)$ 35.65(q, $CF_2\underline{C}H_2C(=CH_2)$, $J_{C,P}$= 21.5Hz, $J_{C,F}$= 36.9Hz), 122.7(td, $\underline{C}F_2$, $J_{C,P}$= 204.9 Hz, $J_{C,F}$= 271.8 Hz), 132.9(s, $C=\underline{C}H_2$), 171.8(C=O); m/z(-ve FAB) 215(MH^-, 35%), 197(20), 177(12), 159(10).

[4,4-Difluoro-4-(diethoxyphosphinyl)-2-iodomethyl] butanoic acid (69b)

2-[2',2'-Difluoroethyl-2'-(diethoxyphosphinyl)] propenoic acid (**68**)(1.66mmol; 0.45g) was stirred with TMSI at room temperature for 30min. Then the excess silylating reagent was removed under reduced pressure to give the trisilylated ester, which was dissolved in ether (50ml) and hydrolysed with water (3x10ml) to give the product in 53% yield; $\nu_{max}(D_2O)/cm^{-1}$ 3831, 3418, 2942, 2508, 1711(C=O), 1434, 1353, 1209(P=O), 1061, 924; $\delta_H(CDCl_3)$ 1.28(m, $CF_2C\underline{H}_2$), 2.03(m, $CF_2CH_2C\underline{H}(CH_2I)$), 2.29(m, $CF_2CH_2CH(C\underline{H}_2I)$; m/z(-ve FAB) 343($MH^-$, 30%)

[4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (70)

To a solution of [(diethoxyphosphinyl) difluoromethyl] zinc bromide (1.30g; 3.90mmol) in 3ml of dry THF, was added a catalytic amount of cuprous iodide(0.2g). Then, 2-bromoacrylic acid (0.71g; 4.70mmol), dissolved in 3ml of dry THF was added dropwise at room temperature, and the mixture was stirred for 4 days. The mixture was filtered and poured into 10ml of brine and extracted with ether (3x10ml). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified using column chromatography on silica gel with

chloroform/methanol/acetic acid (95:4:1) as the eluent to give the product in 23% yield; R_f= 0.46($CHCl_3$:MeOH:AcOH 90:8:2); ν_{max}(liquid film)/cm^{-1} 3459(COOH), 3057, 2981, 1739(C=O), 1596, 1489, 1446, 1373, 1243(P=O), 1174, 1046, 941, 904, 846, 758, 704 cm^{-1}; δ_H($CDCl_3$) 1.39(t, $C\underline{H}_3CH_2OP$, J= 7.05Hz), 2.59-2.83(m, $CF_2C\underline{H}H$), 3.09-3.34(m, $CF_2CH\underline{H}$), 4.25-4.36(m, $CH_3C\underline{H}_2OP$), 4.55(dd, $CH_2C\underline{H}Br$, $J_{2,3b}$= 4.39Hz, $J_{2,3a}$= 9.28Hz); δ_C($CDCl_3$) 16.2(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5.5Hz), 34.9(s, $CF_2CH_2\underline{C}H(Br)$), 39.3(dd, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}$= 36.35Hz, $J_{C,P}$= 19.85Hz), 65.5(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 8.9Hz), 118.7(td, $\underline{C}F_2$, $J_{C,P}$= 219.2Hz, $J_{C,F}$= 262.2Hz), 171.6(s, C=O); δ_F($CDCl_3$) -112.1(dddd, $J_{F,F}$= 301.7Hz, $J_{F,P}$= 105.2Hz, $J_{3b,F}$= 25.4Hz, $J_{3a,F}$= 12.7Hz, 1F), -113.2(dddd, $J_{F,F}$= 301.7Hz, $J_{F,P}$= 105.7Hz, $J_{3b,F}$= 25.5Hz, $J_{3a,F}$= 11.6Hz, 1F); δ_P($CDCl_3$) 5.08(t, ^1H decoupled, $J_{P,F}$= 104Hz; m, ^1H coupled, $J_{P,3a}$= $J_{P,3b}$= 4.03Hz); m/z(C.I.) 339,341(MH^+, 98%) 321,323(M^+-OH, 20), 293,295(M^+-CO_2H, 7). Anal. Calcd. for $C_8H_{14}BrF_2O_5P$: C, 28.3; H, 4.2; Found: C, 28.6; H, 4.3.

E-[4,4-Difluoro-4-(diethoxyphosphinyl)] but-2-enoic acid (71)

To the solution of [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**) (0.49g; 1.44mmol) in 6ml of CH_2Cl_2(dry) was added dropwise DBU (0.25ml; 2.88mmol) at 0°C. Then the solution was allowed to warm to room temperature, and it was left stirring overnight. The solution was acidified with $KHSO_4$(1N) to pH 2.0, and it was washed with brine and extracted with CH_2Cl_2. The product was purified using column chromatography on silica gel with chloroform/methanol/acetic (95:4:1) as the eluent to give the product as an amber liquid in 12.5% yield. Rf= 0.55 ($CHCl_3$:MeOH:AcOH 90:8:2), ν_{max}(liquid film)/cm^{-1} 3423, 2917(COOH), 2360, 1722(C=O), 1641(C=C), 1443, 1260(P=O), 1186, 1106, 1023; δ_H($CDCl_3$) 1.38(t, $C\underline{H}_3CH_2OP$, J= 7.15Hz), 4.23-4.36(m, $CH_3C\underline{H}_2OP$), 6.40(dq, $CF_2CH{=}C\underline{H}$, $J_{2\alpha,3\beta}$= 15.8Hz, $J_{2\alpha,F}$= 5.31Hz, $J_{2\alpha,P}$= 2.57Hz), 6.92(dtd, $CF_2C\underline{H}{=}CH$, $J_{3\beta,2\alpha}$= 15.8Hz, $J_{3\beta,F}$= 12.7Hz, $J_{3\beta,P}$= 1.95Hz), 9.99(s, broad, $COO\underline{H}$); δ_C($CDCl_3$) 16.3(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$=

5.5Hz), 65.4(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 6.6Hz), 117.9(td, $\underline{C}F_2$, $J_{C,F}$= 260.0Hz, $J_{C,P}$= 218.2Hz), 127.9(q, $CF_2CH=\underline{C}H$, $J_{C,P}$=$J_{C,F}$= 7.0Hz), 136.3(td, $CF_2\underline{C}H=CH$, $J_{C,P}$= 13.2Hz, $J_{C,F}$= 22.05Hz), 167.6(s, C=O); m/z(C.I.) 259(MH^+, 259.0547 $C_8H_{13}O_5F_2P$ requires 259.0547, 100%), 213(M^+-COOH, 3).

Z-[4,4-Difluoro-4-(diethoxyphosphinyl) but-2-enoic acid (72)

To the solution of [(diethoxyphosphinyl) difluoromethyl] zinc bromide (1.25g; 3.75mmol) in 6ml of dry THF was added, under N_2, a catalytic amount of cuprous bromide (0.2g). Then cis-3-chloroacrylic acid (0.48g; 4.50mmol) was added, and the mixture was stirred for 24h at room temperature. The mixture was filtered and poured into 10ml of brine and extracted with ether (3x10ml). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified using column chromatography on silica gel with chloroform/methanol/acetic acid (95:4:1) as the eluent to give the product in 20% yield; R_f= 0.35 ($CHCl_3$:MeOH:AcOH 90:8:2); ν_{max}(liquid film)/cm^{-1} 3417, 2989(COOH), 2571, 1731(C=O), 1657(C=C), 1620, 1479, 1396, 1254(P=O), 1164, 1098, 1024, 955, 813, 758; δ_H($CDCl_3$) 1.41(t, $C\underline{H}_3CH_2OP$, J= 7.05Hz), 4.29-4.40(m, $CH_3C\underline{H}_2OP$), 6.01(dtd, $CF_2C\underline{H}=CH$, $J_{3\beta,2\alpha}$= 12.8Hz, $J_{3\beta,F}$= 12.7Hz, $J_{3\beta,P}$= 1.94Hz), 6.37(dq, $CF_2CH=C\underline{H}$, $J_{2\alpha,3\beta}$= 12.9Hz, $J_{2\alpha,F}$= 2.47Hz, $J_{2\alpha,P}$= 2.47Hz); δ_C($CDCl_3$) 16.2(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5,5Hz), 66.2(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 6.6Hz), 116.1(td, $\underline{C}F_2$, $J_{C,F}$= 261.7Hz, $J_{C,P}$= 214.9Hz), 126.9(td, $CF_2\underline{C}H=CH$, $J_{C,P}$= 13.6Hz, $J_{C,F}$= 23.9Hz), 129.9(q, $CF_2CH=\underline{C}H$, $J_{C,P}$=$J_{C,F}$= 7.2Hz), 166.1(s, C=O); m/z(C.I.) 259(MH^+, 259.0547 $C_8H_{13}O_5F_2P$ requires 259.0547, 100%), 241(M^+-OH, 35), 213(M^+-COOH, 20); Anal. Calcd. for $C_8H_{13}F_2O_5P$: C, 37.2; H, 5.1; Found: C, 37.2; H, 5.4.

Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoate (73)

To the solution of [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**) (0.0035 mmol; 1.09g) in 25 ml of dry methanol $HCl_{(gas)}$ was added to the solution until no more was absorbed. The $HCl_{(aq)}$ resulting from the reaction and the excess of methanol was removed URP. The product was purified using column chromatography on silica gel with ethyl acetate/petrol (b.p. 60-80°C) (3:7) as the eluent, giving a pale oil in 76.4% yield; Rf= 0.48(petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2987, 1749 (C=O), 1439, 1371, 1276 (P=O), 1209, 1162, 1020, 981, 796, 761, 573; $\delta_H(CDCl_3)$ 1.39(t, $C\underline{H}_3CH_2O$, $J_{H,H}$= 7.05Hz), 2.62-2.82 (m, $CF_2C\underline{H}H$), 3.13-3.27 (m, $CF_2CH\underline{H}$), 3.81 (s, $OC\underline{H}_3$), 4.23-4.34 (m, $CH_3C\underline{H}_2O$), 4.57 (dd, $CF_2CH_2C\underline{H}(Br)$, $J_{2,3a}$= 4.12 Hz, $J_{2,3b}$= 9.61Hz); $\delta_C(CDCl_3)$ 15.72 (d, $\underline{C}H_3CH_2O$, $J_{C,P}$= 5.5 Hz), 34.18 (d, $CF_2CH_2\underline{C}H(Br)$, $J_{C,F}$= 6.6Hz), 38.9 (q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,P}$= 20.95 Hz, $J_{C,F}$= 36.35Hz), 52.6 (s, $O\underline{C}H_3$), 64.3 (d, $CH_3\underline{C}H_2O$, $J_{C,P}$ = 6.6 Hz), 118.2 (td, $\underline{C}F_2$, $J_{C,P}$= 216.0Hz, $J_{C,P}$= 259.0Hz), 168.8 (s, C=O); $\delta_F(CDCl_3)$ -112.3 (dddd, $J_{F,F}$= 301.7Hz, $J_{F,P}$= 103.5Hz, $J_{3b,F}$= 22.5Hz, $J_{3a,F}$= 15.1Hz, 1F), -113.4(dddd, $J_{F,F}$= 301.7Hz, $J_{F,P}$= 103.1Hz, $J_{3b,F}$= 22.5Hz, $J_{3a,F}$= 13.9Hz, 1F); $\delta_P(CDCl_3)$ 5.15(t, ^1H decoupled, $J_{P,F}$= 104Hz) m/z(C.I.) 353.355 (MH^+, 100%), 321.323 (M^+ -CO_2H, 8), 275 (20); m/z(E.I.) 352; 354 (M^+, 353.98663 $C_9H_{16}O_5F_2BrP$ requires 353.98272, 6%).

Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butanoate (74)

A solution of methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoate (**73**) (5.74 mmol; 2.02 g) in 15 ml of acetone and 15 ml of water was treated with sodium azide (9.23 mmol; 0.6g), and stirred at room temperature for 24h. The reaction mixture was concentrated under reduced pressure and poured into EtOAc. The mixture was extrated with ethyl acetate (3x20ml). The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed (petrol-ethyl

acetate 1:1) to yield the title compound as a pale oil in 60.1% yield; Rf= 0.65 (petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2986, 2123 (N_3), 1749 (C=O), 1272 (P=O), 1019; δ_H($CDCl_3$) 1.40(t, $C\underline{H}_3CH_2O$, $J_{H,H}$= 6.87 Hz), 2.34-2.51 (m, $CF_2CH\underline{H}$), 2.67-2.83 (m, $CF_2C\underline{H}H$), 3.83 (s, OCH_3), 4.26-4.35 (m, $CH_3C\underline{H}_2O$ and $CF_2CH_2C\underline{H}(N_3)$); δ_C($CDCl_3$) 16.2(d, $\underline{C}H_3CH_2O$, $J_{C,P}$= 3.6Hz), 35.3 (td, $CF_2\underline{C}H_2CH(N_3)$), $J_{C,P}$= 15.97Hz, $J_{C,F}$= 20.67Hz), 53.04 (s, $O\underline{C}H_3$), 55.84 (q, $CF_2CH_2\underline{C}H(N_3)$, $J_{C,P}$= 5.5 Hz, $J_{C,F}$= 9.2Hz), 64.9 (d, $CH_3\underline{C}H_2O$, $J_{C,P}$= 5.5Hz), 118.79 (td, $\underline{C}F_2$, $J_{C,P}$= 216.9Hz, $J_{C,F}$= 261.9 Hz), 169.41 (s, C=O); δ_F($CDCl_3$) -111.5 (dddd, $J_{F,F}$= 300.7Hz, $J_{F,P}$= 103.5Hz, $J_{3b,F}$= 25.5Hz, $J_{3a,F}$= 11.6Hz, 1F), -112.9(dddd, $J_{F,F}$= 300.7Hz, $J_{F,P}$= 104.7Hz, $J_{3b,F}$= 24.8Hz, $J_{3a,F}$= 12.7Hz, 1F); δ_P($CDCl_3$) 5.50(t, ^1H decoupled, $J_{P,F}$= 104Hz); m/z(C.I.) 316 (MH^+, 316.0874 $C_9H_{16}O_5F_2N_3P$ requires 316.0874, 100%), 288 (12), 268(40), 228(15); m/z (E.I.) 316 (M^+, 2%), 284 (M^+-OMe, 2), 228 (30), 200 (34), 172 (100).

Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butanoate hydrochloride salt (75)

Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butanoate (**74**) (2.07 mmol; 0.65g) in methanol (15 ml) and HCl (1.0 ml) was hydrogenolyzed over 5%-Pd/C in the usual manner. The mixture was stirred overnight and then filtered through celite. The solvents were evaporated under reduced pressure, then the reaction mixture was dissolved in water (10 ml) and it was lyophilised to yield the title compound as a cream solid (0.52 g; 76.4% yield); R_f= 0.20($CHCl_3$-MeOH 1:1); ν_{max}(liquid film)/cm^{-1} 3435 (OH), 2984, 1755 (C=O), 1601 (-NH_3^+), 1514, 1442, 1371, 1245 (P=O), 1161, 1020, 794, 751; δ_H(D_2O) 1.45 (t, $C\underline{H}_3CH_2O$, J= 7.05Hz), 2.89-3.09 (m, $CF_2C\underline{H}_2$), 3.93 (s,OCH_3), 4.38-4.49 (m,$CH_3C\underline{H}_2O$), 4.68 (dd, $CH_2C(\underline{H})NH_3^+Cl^-$, $J_{2,3a}$= 4.03Hz, $J_{2,3b}$= 7.51Hz); δ_C(MeOD) δ 17.0 ppm (d, $\underline{C}H_3CH_2O$, $J_{C,P}$= 5.5Hz), 35.4 (q, $CF_2\underline{C}H_2C(H)NH_3^+Cl^-$), $J_{C,P}$= 19.1Hz, $J_{C,F}$= 38.4Hz), 48.7 (s, $CF_2CH_2\underline{C}(H)NH_3^+Cl^-$),

54.7 (s, O$\underline{C}H_3$), 67.3 (d, $CH_3\underline{C}H_2O$, $J_{C,P}$= 7.7Hz), 121.2 (td, $\underline{C}F_2$, $J_{C,P}$= 219.5Hz, $J_{C,F}$= 261.4Hz), 169.6 (s, C=O); m/z(+ve FAB) 290 (MH^+, 290.0980 $C_9H_{18}O_5F_2NP$ requires 290.0969, 100%), 262(5), 210(10).

[4,4-Difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (76)

The methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butanoate hydrochloride salt (75) (0.86; 2.63 mmol) was stirred with TMSI in excess (2.5 ml) without solvent at 60°C for 07 days. The excess of silylating reagent and ethyl iodide were removed URP to give the disilylated ester, which was dissolved in ether (30 ml), and then treated with water (30 ml) to provide the corresponding free amine and phosphonic acid, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (76). The compound (76) was lyophilised and purified by washing with methanol. The impurities stayed in the methanol, and the compound (76) precipitated as a white solid (0.472 g; 82% yield); m.p.= 220°C; $\nu_{max}(D_2O)/cm^{-1}$ 3423 (OH), 2527 (NH_3^+), 1746 (C=O), 1449, 1209 (P=O); $\delta_H(D_2O)$ 2.64-2.80 (m, $CF_2C\underline{H}_2CH(NH_3^+)$), 4.32(d, $CF_2CH_2C\underline{H}(NH_3^+)$, $J_{H,H}$= 6.77Hz); $\delta_C(D_2O)$ δ 33.4 ppm (q, $CF_2\underline{C}H_2CH(NH_3^+)$, $J_{C,P}$= 18.9Hz, $J_{C,F}$= 36.6Hz), 47.5 (s, $CF_2CH_2\underline{C}H(NH_3^+)$), 120.5 (td, $\underline{C}F_2$, $J_{C,P}$= 195.5Hz, $J_{C,F}$= 259.2 Hz), 170.7 (s, C=O); m/z(+ve FAB) 220 (MH^+, 35%), 191(55), 152(100), 138(95), 122(40); m/z(-ve FAB) 218 (MH^-, 218.0039 $C_4H_8F_2NO_5P$ requires 218.0030, 75%), 184(30), 153(100); Anal. Calcd for $C_4H_8F_2NO_5P$ + 1.5 H_2O: C, 19.50; H, 4.47; N, 5.70; Found: C, 19.70, H, 4.31; N. 5.70.

Bornyl [(4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butyrate (78)

To a stirred solution of [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (70)(3.42g; 10.11mmol) in anhydrous CH_2Cl_2 (30ml) was added [(1S)-endo]-(-)-borneol (1.64g; 10.62mmol) and DMAP (10 mol%). DCC (2.19g;

10.62mmol) was added to the reaction mixture at 0°C. The mixture was then stirred for 5 h, gradually reaching room temperature. Precipitated urea was then filtered off and the filtrate concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and, if necessary, filtered free of any further precipated urea. Column chromatography (petrol-ethyl acetate 9:1) yielded the desired ester as a colourless oil (3.76g; 78%); R_f= 0.59 (petrol-ethyl acetate 7:3); υ_{max}(liquid film)/cm^{-1} 2955, 2876, 1743(C=O), 1666, 1528, 1479, 1453, 1426, 1390, 1274(P=O), 1159, 1113, 1020, 979; $\delta_H(CDCl_3)$ 0.79, 0.80, 0.81, 0.84(s, $C\underline{H}_3$ 8', 9' 10'), 0.97(dd, $C\underline{H}_2$ 2', $J_{H(2'),H(2')}$= 13.92Hz, $J_{H(2'),H(1')}$= 3.3Hz), 1.14-1.25(m, $C\underline{H}_2$ 5'), 1.32(t, $C\underline{H}_3CH_2OP$, $J_{H,H}$= 7.15Hz), 1.63(t, $C\underline{H}$ 3', $J_{H(3'),H(4')}$= $J_{H(3'),H(2')}$= 4.4Hz), 1.67-1.79(m, $C\underline{H}_2$ 4'), 1.86-1.95(m, $C\underline{H}_2$ 4'), 2.23-2.39(m, $C\underline{H}_2$ 2'), 2.57-2.79(m,$CF_2C\underline{H}_2CH(Br)$), 3.13-3.29(m, $CF_2C\underline{H}_2CH(Br)$), 4.18-4.37(m, $CH_3C\underline{H}_2OP$), 4.47-4.69(m, $C\underline{H}$ 1'), 4.85-5.31(m, $CF_2CH_2C\underline{H}(Br)$); $\delta_C(CDCl_3)$ 12.3(C-10'), 15.4(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5.5Hz), 17.8(C-8'), 18.6(C-9'), 26.0(C-4'), 26.9(C-5'), 34.9(s, $CF_2CH_2\underline{C}H(Br)$), 35.0(C-2'), 38.4(q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}$= 35.2Hz, $J_{C,P}$= 19.8Hz), 43.8(C-3'), 47.1(C-7'), 48.1(C-6'), 63.9(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 6.6Hz), 81.0(C-1'), 117.9(td, $\underline{C}F_2$, $J_{C,P}$= 216.0Hz, $J_{C,F}$= 261.0Hz), 168.0(s, C=O). m/z (C.I.) 475,477(MH^+, 18%), 339,341(100), 321,323(12), 293,295(3). Anal. Calcd. for $C_{18}H_{30}BrF_2O_5P$: C, 45.5; H, 6.4; Found: C, 45.9; H, 6.5.

Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butyrate (80)

A solution of bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butyrate (78)(3.76g; 7.91mmol) in 20 ml of acetone and 10 ml of water was treated with sodium azide (0.57g; 8.70mmol) and stirred at 40°C for 10 h. Then, the reaction mixture was concentrated under reduced pressure and extracted with ethyl acetate (3x 15ml). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Column chromatography (petrol-ethyl acetate 4:1) yielded the title compound as a pale oil (2.26g; 65%); R_f= 0.28 (petrol-ethyl acetate 7:3); υ_{max}(liquid film)/cm^{-1} 2956,

2110(N_3), 1743(C=O), 1454, 1391, 1273(P=O), 1194, 1020, 980; δ_H($CDCl_3$) 0.86, 0.87, 0.89, 0.91(s, $C\underline{H}_3$ 8', 9' 10'), 1.04(dt, $C\underline{H}_2$ 2', $J_{H(2'),H(2')}$= 13.9Hz, $J_{H(2'),H(1')}$= $J_{H(2'),(H3')}$= 3.7Hz), 1.23-1.31(m, $C\underline{H}_2$ 5'), 1.40(t, $C\underline{H}_3CH_2OP$, $J_{H,H}$= 7.14Hz), 1.72(t, $C\underline{H}$ 3', $J_{H(3'),H(4')}$= $J_{H(3'),H(2')}$= 4.4Hz), 1.76-1.82(m, $C\underline{H}_2$ 4'), 1.89-1.99(m, $C\underline{H}_2$ 4'), 2.30-2.55(m, $CF_2C\underline{H}_2CH(N_3)$ and $C\underline{H}_2$ 2'), 2.65-2.85(m, $CF_2C\underline{H}_2CH(N_3)$), 4.25-4.36(m, $CF_2CH_2C\underline{H}(N_3)$ and $CH_3C\underline{H}_2OP$), 4.97-5.01(m, CH 1'); δ_C($CDCl_3$) 13.4(C-10'), 16.3(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 5.5Hz), 18.8(C-8'), 19.6(C-9'), 27.1(C-4'), 27.9(C-5'), 35.3(q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}$= 20.1Hz, $J_{C,P}$= 16.5Hz), 36.6(C-2'), 44.7(C-3'), 47.9(C-7'), 48.9(C-6'), 56.1(s, $CF_2CH_2\underline{C}H(Br)$), 64.8(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 7.3Hz), 82.7(C-1'), 120.2(td, $\underline{C}F_2$, $J_{C,P}$= 216.9Hz, $J_{C,F}$= 261.0Hz), 169.2(s, C=O); δ_F($CDCl_3$) -111.4 (ddddd, $J_{F,F}$= 300.5Hz, $J_{F,P}$= 104.2Hz, $J_{3b,F}$= 25.0Hz, $J_{3a,F}$= 19.7Hz, $J_{2,F}$= 12.7Hz, 1F), -112.5(ddddd, $J_{F,F}$= 300.5Hz, $J_{F,P}$= 104.9Hz, $J_{3b,F}$= 34.4Hz, $J_{3a,F}$= 23.9Hz, $J_{2,F}$= 13.9Hz, 1F); δ_P($CDCl_3$) 5.6(t, ^1H decoupled, $J_{P,F}$= 105Hz; m, ^1H coupled, $J_{P,3a}$= $J_{P,3b}$= 6.9Hz). m/z (C.I.) 438(MH^+, 18%), 302(100), 228(8), 210(10), 137(40). Anal. Calcd. for $C_{18}H_{30}F_2N_3O_5P$: C, 49.3; H, 6.9; N, 9.6; Found: C, 49.6; H, 7.1; N, 9.4.

N-[4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butyryl urea (79)

This compound was formed as a side product in the synthesis of bornyl [(4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butyrate (**78**) when [4,4-difluoro-4-(dietoxyphosphinyl)-2-bromo] butanoic acid (**70**) was treated with [(1S)-endo]-(-)-borneol. It was a rearrangement of the O-acylisourea intermediate. Compound (**79**) was unseparable from compound (**78**).

The analysis of compound (**79**) was only possible when the reaction of compound (**70**) with [(1S)-endo]-(-)-borneol was carried out in the presence of a small amount of polar solvent such as methanol or ethyl acetate, facilitating the rearrangement of O-acylisourea to N-acylurea.

To a stirred solution of [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**70**) (2.27g; 6.71mmol) at 0°C in THF (10ml) and MeOH(1 ml) was added [(1S)-endo]-(-)-borneol (1.2g; 7.78mmol) and DCC(1.52g; 7.38mmol). It was stirred overnight, then the precipitate urea was filtered off and the filtrate concentrated under reduced pressure. Column chromatrography (petrol-ethyl acetate 8:2) yielded the N-acylurea as a white solid; m.p.= 89.0 ^{O}C; R_f= 0.39 (petrol-ethyl acetate 7:3); $\nu_{max}(CDCl_3)/cm^{-1}$ 3285, 2935, 2858, 2252($CDCl_3$), 1706(C=O), 1660(C=O), 1531, 1451, 1395, 1342, 1264(P=O), 1232, 1158, 1030, 908, 735, 649; $\delta_H(CDCl_3)$ 1.10-1.31(m, C$\underline{H}_2$ cyclohexyl), 1.38(t, C$\underline{H}_3$CH$_2$OP partially obscured by cyclohexyl, $J_{C,P}$= 3.6Hz), 1.42-2.05(m, C$\underline{H}_2$ cyclohexyl), 2.58-2.70(m, CF$_2$C$\underline{H}_2$CH(Br)), 3.40-3.72(m, CF$_2$C$\underline{H}_2$CH(Br) and C$\underline{H}$ cyclohexyl), 4.10-4.21(m, C$\underline{H}$ cyclohexyl), 4.24-4.32(m, CH$_3$C$\underline{H}_2$OP), 4.90(d, CF$_2$CH$_2$C$\underline{H}$(Br), $J_{H,H}$= 8.79Hz), 7.12(s, NH); $\delta_C(CDCl_3)$= 16.4(d, C$\underline{H}_3$CH$_2$OP, $J_{H,H}$= 5.6Hz), 24.7, 25.4, 25.8, 25.9, 29.6, 30.6, 32.0 and 32.5 (cyclohexyl $\underline{C}H_2$), 34.8 and 34.9 (cyclohexyl $\underline{C}$H), 40.1(q, CF$_2\underline{C}$H$_2$CH(Br), $J_{C,P}$= 16.5Hz, $J_{C,F}$= 22.1Hz), 50.1(s, CF$_2$CH$_2\underline{C}$H(Br)), 65.2(d, CH$_3\underline{C}$H$_2$OP, $J_{C,P}$= 7.3Hz), 118.5(td, $\underline{C}F_2$, $J_{C,P}$= 215.1Hz, $J_{C,F}$= 261.0Hz), 152.7(C=O), 165.7(C=O); m/z(C.I.) 545;547(MH^+, 10%), 420,422(55). Anal. Calcd. for $C_{21}H_{36}BrF_2N_2O_5P$: C, 46.3; H, 6.7; N, 5.1; Found: C, 46.4; H, 6.9; N, 5.0.

N-(4,4-Difluoro-4-(diethoxyphosphinyl)-2-azido) butyryl urea (81)

A solution of N-[4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butyryl urea (**79**)(0.25g; 0.45mmol) in 20ml of acetone and 10ml of water was treated with sodium azide (0.1g) and stirred at 40°C for 10 hours. Then, the reaction mixture was concentrated under reduced pressure and extracted with ethyl acetate (3x15ml). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Column chromatrography (petrol-ethyl acetate 7:3) yielded the compound (**81**) as a white solid, which was recrystallized from ethyl acetate to give crystals of suitable

quality for X-ray; R_f= 0.73 (petrol-ethyl acetate 1:1); $\nu_{max}(CHCl_3)/cm^{-1}$ 3019, 2935, 2858, 2107(N_3), 1706(C=O), 1660(C=O), 1506, 1398, 1353, 1216(P=O), 1029, 761, 669; $\delta_H(CDCl_3)$ 1.10-1.35(m, C$\underline{H}_2$ cyclohexyl), 1.40(t, C$\underline{H}_3$CH$_2$OP partially obscured by cyclohexyl, $J_{H,H}$= 3.60Hz), 1.54-1.98(m, C$\underline{H}_2$ cyclohexyl), 2.35-2.52(m, $CF_2C\underline{H}_2CH(N_3)$), 2.99-3.16(m, $CF_2C\underline{H}_2CH(N_3)$), 3.63-3.72(m, C$\underline{H}$ cyclohexyl), 4.09-4.15(m, C$\underline{H}$ cyclohexyl), 4.23-4.31(m, $CH_3C\underline{H}_2OP$ and $CF_2CH_2C\underline{H}(N_3)$), 6.78(s, NH); $\delta_C(CDCl_3)$= 16.3(d, C$\underline{H}_3$CH$_2$OP, $J_{C,P}$= 5.5Hz), 24.5, 25.3, 25.5, 25.8, 30.2, 31.1, 31.9 and 32.4 (cyclohexyl $\underline{C}H_2$), 35.6(q, $CF_2\underline{C}H_2CH(N_3)$, $J_{C,P}$= 16.6Hz, $J_{C,F}$= 22.1Hz), 50.2(s, $CF_2CH_2\underline{C}H(N_3)$), 54.4 and 54.98(cyclohexyl $\underline{C}$H), 65.1(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 5.5Hz), 118.5(td, $\underline{C}F_2$, $J_{C,P}$= 215.1Hz, $J_{C,F}$= 261.0Hz), 152.5(C=O), 165.7(C=O); m/z(C.I.) 508(MH^+, 8%), 465(30), 383(100). Anal. Calcd. for $C_{21}H_{36}F_2N_5O_5P$: C, 49.7; H, 7.2; N, 13.8; Found: C, 49.9; H, 7.4; N, 13.6.

Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloride salt (82 R and 82 S) and Bornyl [4,4-difluoro-4-(ethoxy-hydroxyphosphinyl)-2-amino] butyrate hydrochloride salt (83 R and 83 S).

Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butyrate (**80**) (1.35g; 31mmol) in methanol (20ml) and HCl (9.0ml) was hydrogenolyzed over 5% Pd/C in the usual manner. The mixture was stirred overnight and then filtered through celite. The solvents were evaporated under reduced pressure, then the reaction was dissolved in water (20ml) and lyophilised. TLC indicated four products: bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloride salt (**82 R**) and (**82 S**) in 50.6% yield, and bornyl [4,4-difluoro-4-(ethoxyhydroxyphosphinyl)-2-amino] butyrate hydrochloride salt (**83 R**) and (**83 S**) in 14.6% yield. These four products were separated by column chromatography (chloroform-methanol 99:1 to 95:5).

The first band eluted (Rf= 0.75, chloroform-methanol 90:10), was collected and evaporated to afford *bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate*

hydrochloric salt (**82 R**); ν_{max}(liquid film)/cm^{-1} 3743, 2956, 2361, 1733 (C=O), 1650, 1454, 1389, 1274 (P=O), 1188, 1021; δ_H($CDCl_3$) 0.89, 0.90, 0.93, 0.96(s, C$\underline{H}_3$ 8', 9' 10'), 1.06(dd, C$\underline{H}_2$ 2', $J_{H(2'),H(2')}$= 13.83Hz, $J_{H(2'),H(1')}$= 3.2Hz), 1.16(t, C$\underline{H}$ 3', $J_{H(3'),H(4')}$= $J_{H(3'),H(2')}$= 7.15Hz) 1.25-1.37(m, C$\underline{H}_2$ 5'), 1.43(t, C$\underline{H}_3$CH$_2$OP, $J_{H,H}$= 7.05Hz), 1.69-1.99(m, C$\underline{H}_2$ 4'), 2.00-2.36(m, C$\underline{H}_2$ 4'), 2.41-2.76(m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$ and C$\underline{H}_2$ 2'), 3.73(ddd, $CF_2CH_2C\underline{H}(NH_3^+Cl^-)$, $J_{2,3a}$= 4.87Hz, $J_{2,3b}$= 7.63Hz, $J_{2,F}$= 3.35Hz), 4.27-4.38(m, $CH_3C\underline{H}_2OP$), 4.96-4.99(m, C$\underline{H}$ 1'); δ_C($CDCl_3$) 13.3(C-10'), 16.3(s, $\underline{C}H_3CH_2OP$), 18.7(C-8'), 19.6(C-9'), 27.0(C-4'), 27.9(C-5'), 37.3(q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}$= 35.8Hz, $J_{C,P}$= 17.5Hz), 41.9(C-2'), 44.8(C-3'), 47.7(C-7'), 48.7(C-6'), 55.2(s, $CF_2CH_2\underline{C}H(NH_3^+Cl^-)$), 64.5(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 5.5Hz), 80.8(C-1'), 119.6(td, $\underline{C}F_2$, $J_{C,P}$= 215.1Hz, $J_{C,F}$= 261.0Hz), 174.1(s, C=O). m/z(-ve FAB) 410.2(MH^-, 100%), 398.1(12), 302.1(22), 274.1(M^+-$C_{10}H_{17}$, 10).

The second band eluted (Rf= 0.65, chloroform-methanol 90:10), was collected and evaporated to give *bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloric salt* (**82 S**); ν_{max}(liquid film)/cm^{-1} 3743, 2954, 2361, 1735(P=O), 1650, 1454, 1391, 1271(P=O), 1165, 1022; δ_H($CDCl_3$) 0.84, 0.85, 0.88, 0.91(s, C$\underline{H}_3$ 8', 9' 10'), 0.99(dddd, C$\underline{H}_2$ 2', $J_{H(2'),H(2')}$= 13.7Hz, $J_{H(2'),H(1')}$= 3.5Hz $J_{H(2'),H(3')}$= 5.31Hz), 1.19-1.32(m, C$\underline{H}_2$ 5'), 1.39(t, C$\underline{H}_3$CH$_2$OP, $J_{H,H}$= 7.05Hz), 1.69(t, C$\underline{H}$ 3', $J_{H(3'),H(4')}$= $J_{H(3'),H(2')}$= 4.4Hz), 1.74-1.81(m, C$\underline{H}_2$ 4'), 1.85-1.97(m, C$\underline{H}_2$ 4'), 2.24-2.49(m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$ and C$\underline{H}_2$ 2'), 2.58-2.79(m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$), 3.79(dd, $CF_2CH_2C\underline{H}(N_3)$, $J_{2,3a}$= 4.58 Hz, $J_{2,3b}$= 7.69 Hz) 4.24-4.35(m, $CH_3C\underline{H}_2OP$), 4.89-4.94(m, C$\underline{H}$ 1'); δ_C($CDCl_3$) 13.4(C-10'), 16.3(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 3.7Hz), 18.7(C-8'), 19.6(C-9'), 27.0(C-4'), 27.9(C-5'), 36.5(C-2'), 38.6(q, $CF_2\underline{C}H_2CH(NH_3^+Cl^-)$, $J_{C,F}$= 34.1Hz, $J_{C,P}$= 17.5Hz), 44.8(C-3'), 47.8(s, $CF_2CH_2\underline{C}H(NH_3^+Cl^-)$), 48.8(C-7'), 49.3(C-6'), 64.6(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 5.5Hz), 81.2(C-1'), 120.2(td, $\underline{C}F_2$, $J_{C,P}$= 215.0Hz, $J_{C,F}$= 260.6Hz), 173.9(s, C=O). m/z(+ve FAB) 412.3(MH^+, 100%), 276.1(60), 174.1(30).

The third band eluted (Rf= 0.28, chloroform-methanol 90:10), was collected and evaporated to give *bornyl [4,4-difluoro-4-(ethoxyhydroxyphosphinyl)-2-amino] butyrate hydrochloric salt* (**83 R**); $\nu_{max}(CDCl_3)/cm^{-1}$ 3743, 3020, 2927, 2361, 1728(C=O), 1650, 1496, 1215(P=O), 762; $\delta_H(CDCl_3)$ 0.85, 0.87, 0.88, 0.90(s, $C\underline{H}_3$ 8', 9' 10'), 0.93-1.13(m, $C\underline{H}_2$ 2' and $C\underline{H}_2$ 5'), 1.28(t, $C\underline{H}$ 3', $J_{H(3'),H(4')}= J_{H(3'),H(2')}=$ 7.23Hz), 1.38(t, $C\underline{H}_3CH_2OP$, $J_{H,H}=$ 7.15Hz), 1.69-1.75(m, $C\underline{H}_2$ 4'), 1.98(m, broad signal, $C\underline{H}_2$ 4'), 2.30-2.38(m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$, 2.84-2.87(m, $C\underline{H}_2$ 2'), 3.04-3.25(m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$), 3.9(broad overlapped signal, $CF_2CH_2C\underline{H}(NH_3^+Cl^-)$), 4.07-4.13(m, $CH_3C\underline{H}_2OP$), 4.94-5.14(m, $C\underline{H}$ 1'); $\delta_C(CDCl_3)$ 11.2(C-10'), 13.4(d, $\underline{C}H_3CH_2OP$, $J_{C,P}=$ 7.4Hz), 18.7(C-8'), 19.6(C-9'), 27.1(C-4'), 27.8(C-5'), 35.2(q, overlapped signal, $CF_2\underline{C}H_2CH(NH_3^+Cl^-)$), 41.6(C-2'), 44.7(C-3'), 47.9(C-7'), 48.9(C-6'), 54.8(s, $CF_2CH_2\underline{C}H(NH_3^+Cl^-)$), 62.9(s, $CH_3\underline{C}H_2OP$), 82.9(C-1'), $\underline{C}F_2$ very weak signal, 167.7(s, C=O); m/z(-ve FAB) 382(MH^-, 100%), 335.1(30), 332.1(24), 182.1(34).

The lower band eluted gave *bornyl [4,4-difluoro-4-(ethoxyhydroxy phosphinyl)-2-amino] butyrate hydrochloric salt* (**83 S**); Rf= 0.18 (chloroform-methanol 90:10); $\nu_{max}(CDCl_3)/cm^{-1}$ 3743, 2958, 2361, 2253, 1745(C=O), 1649, 1455, 1388, 1232(P=O), 1052, 908, 735; $\delta_H(CDCl_3)$ 1.00, 1.03, 1.05(s, $C\underline{H}_3$ 8', 9' 10'), 1.23-1.26(m, $C\underline{H}_2$ 2' and $C\underline{H}_2$ 5'), 1.39(t, $C\underline{H}_3CH_2OP$, $J_{H,H}=$ 7.0Hz), 1.81-2.09(m, $C\underline{H}$ 3' and $C\underline{H}_2$ 4'), 2.50-2.82(m, $C\underline{H}_2$ 4'), 2.50(m, broad signal, $C\underline{H}_2$ 2'), 2.82(m, broad signal, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$), 4.16-4.21(m, $CH_3C\underline{H}_2OP$), 4.42(broad overlapped signal, $CF_2CH_2C\underline{H}(NH_3^+Cl^-)$), 5.14-5.36(m, $C\underline{H}$ 1'); $\delta_C(CDCl_3)$ 13.3(C-10'), 16.5(s, $\underline{C}H_3CH_2OP$), 18.7(C-8'), 19.5(C-9'), 26.9(C-4'), 27.7(C-5'), 29.6(C-2'), 35.7(q, overlapped signal, $CF_2\underline{C}H_2CH(NH_3^+Cl^-)$), 44.7(C-3'), 47.8(C-7'), 48.6(C-6'), 48.8(s, $CF_2CH_2\underline{C}H(NH_3^+Cl^-)$), 63.4(d, $CH_3\underline{C}H_2OP$, $J_{C,P}=$ 5.5Hz), 82.7(C-1'), $\underline{C}F_2$ very weak signal, 170.6(s, C=O); m/z(-ve FAB) 382(MH^-, 100%), 260.1(10), 248.9(5), 190.0(10).

(R,S)[4,4-Difluoro-4-(diethoxyphosphinyl)-2-amino] butanoic acid (76)

(a) *Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloric salt* (**82 R**)(74.8mg; 0.17mmol) and *bornyl [4,4-difluoro-4-(ethoxyhydroxy phosphinyl)-2-amino] butyrate hydrochloric salt* (**83 R**)(30.8mg; 0.073mmol) were stirred with TMSI in excess (0.05ml) and (0.15ml) respectively, without solvent at 70°C for 07 days. The excess of silylating reagent and ethyl iodide were removed under reduced pressure to give the disilylated ester, which was dissolved in ether (20ml), and then treated with water (15ml) to give the (R)[4,4-difluoro-4-(diethoxy phosphinyl)-2-amino] butanoic acid (**76 R**) in 90% yield; Rf= 0.3 (H_2O/NH_3/propanol 1:1:3); $\nu_{max}(D_2O)/cm^{-1}$ 3838, 3430, 2536, 1734(C=O), 1451, 1207(P=O), 1083; $\delta_H(D_2O)$ 2.73-2.87(m, $CF_2C\underline{H}_2CH(NH_2)$), 4.29(overlapped signal, $CF_2CH_2C\underline{H}(NH_2)$; $\delta_C(D_2O)$ 36.0(d, $CF_2\underline{C}H_2CH(NH_2)$, $J_{C,F}$= 14.7Hz), 57.42(s, $CF_2CH_2\underline{C}H(NH_2)$), $\underline{C}F_2$(weak signal), 173.2(s, C=O); m/z(ve +FAB) 220(MH^+, 12%), 192(10), 176(100).

(b) *Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloric salt* (**82 S**)(83.9mg; 0.19mmol) and *bornyl [4,4-difluoro-4-(ethoxyhydroxy phosphinyl)-2-amino] butyrate hydrochloric salt* (**83 S**)(0.12g; 0.27mmol) were stirred with TMSI in excess (0.2ml) and (0.3ml) respectively without solvent at 70°C for 7 days. The excess of silylating reagent and ethyl iodide were removed under reduced pressure to give the disilylated ester, which was dissolved in ether (20ml), and then treated with water (15ml) to give the (S)[4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butanoic acid (**76 S**) in 90% yield; Rf= 0.2 (H_2O/NH_3/propanol 1:1:3); $[\alpha]_D$ = 30.4 (c= 4.6 in water); $\nu_{max}(D_2O)/cm^{-1}$ 3853, 3743, 3425, 2926, 2529, 2362, 1650, 1455, 1206(P=O); $\delta_H(D_2O)$ 2.72-3.11(m, $CF_2C\underline{H}_2CH(NH_2)$), 4.5(dd, $CF_2CH_2C\underline{H}(NH_2)$, $J_{2,3a}$= 3.4Hz, $J_{2,3b}$= 8.9Hz); $\delta_C(D_2O)$ 37.06(td, $CF_2\underline{C}H_2CH(NH_2)$, $J_{C,F}$= 11.5Hz, $J_{C,P}$= 9.0Hz), 51.47(d, $CF_2CH_2\underline{C}H(NH_2)$, $J_{C,F}$= 2.0Hz), $\underline{C}F_2$(weak signal), 174.9(s, C=O); $\delta_F(D_2O)$ -112.2 (dddd, $J_{F,F}$= 290.2Hz, $J_{F,P}$= 91.3Hz, $J_{3b,F}$= 23.1Hz, $J_{3a,F}$= 15.1Hz, 1F),

-113.2(dddd, $J_{F,F}$= 290.2Hz, $J_{F,P}$= 91.3Hz, $J_{3b,F}$= 22.0Hz, $J_{3a,F}$= 16.2Hz, 1F); $\delta_P(CDCl_3)$ 2.8(t, ^1H decoupled, $J_{P,F}$= 91.2Hz; m/z(ve +FAB) 220(MH^+, 12%), 192(10), 176(100).

Methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-hydroxy-2-methyl] propionate (84)

A solution of butyl lithium(4.66mmol, 2.91ml) in hexane was added at 0°C to a stirred solution of diisopropylamine(4.66mmol, 0.65ml) in dry THF(10ml), and stirred for 30min. Then the solution was cooled to -78°C and a solution of diethyl difluoromethyl phosphonate(4.05mmol, 0.7613g) in dry THF(10 ml), pre-cooled at -78°C, was added slowly and the mixture was then stirred for 1h at -78°C. Methyl pyruvate (6.1mmol, 0.55ml) in dry THF(10 ml), pre-cooled at -78°C, was added dropwise and the mixture was stirred at -78°C for 6h, then slowly warmed to room temperature, and left stirring for an additional 2h. Then the reactiom mixture was poured into dry ether (50ml), and the mixture was washed with NH_4Cl (saturated solution, 3x10ml). The organic layer was dried over Na_2SO_4 and concentrated U.R.P.. The product was purified using column chromatography on silica gel with ethyl acetate light petroleum (b.p. 60-80°C) (1:1) as the eluent, to give a colorless oil; R_f= 0.26(petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 3474, 2990, 1747(C=O), 1657, 1265(P=O), 1168, 1022(OCH_3), 983, 862, 798, 750; $\delta_H(CDCl_3)$ 1.38(t, $C\underline{H}_3CH_2OP$, $J_{H,H}$= 7.1Hz), 1.62(t, $CF_2COH(C\underline{H}_3)$, $J_{H,F}$= 1.47 Hz), 3.87(s, OCH_3), 4.01(s, OH); 4.29(m, $CH_3C\underline{H}_2OP$); $\delta_C(CDCl_3)$ 16.2(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 4.4Hz), 19.1(s, C-$\underline{C}H_3$), 53.5(s, OCH_3), 64.8(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 6.6Hz), 117.7(td, CF_2, $J_{C,F}$= 274.3Hz, $J_{C,P}$= 207.1Hz), 171.9(s, C=O); $\delta_F(CDCl_3)$ -115.1(dd, $J_{F,F}$= 306.9Hz, $J_{F,P}$= 98.9Hz, 1F), -118.4(dd, $J_{F,F}$= 306.9Hz, $J_{F,P}$= 102.3Hz, 1F); $\delta_P(CDCl_3)$ 5.1(t, ^1H decoupled, $J_{P,F}$= 101.1Hz; m, ^1H coupled, $J_{P,H}$= 7.74Hz); m/z(E.I.) 290(M^+,2%), 231(M^+-CO_2Me, 68), 187(95), 175(100); m/z(C.I.) 291(M+1, 100%). Anal. Calcd. for $C_9H_{17}F_2O_6$: C, 37.2; H, 5.9; Found: C, 37.2; H, 6.1.

Diethyl difluoromethylphosphonate (46)

A suspension of sodium hydride (80% in mineral oil, 6g, 0.2mol) was washed with 40-60 petrol (2x5ml), and with dry diethyl ether (2x5ml), and then suspended in dry diethyl ether (50ml). A solution of diethyl phosphite (g, 0.1mol) in dry diethyl ether (50 ml) was added dropwise to the solution of sodium hydride with ice/water cooling. When the addition was complete, the white suspension was stirred at room temperature for 2 h, then cooled again with an ice/water bath. Chlorodifluoromethane was added to the reaction mixture until no more was absorbed. The reaction mixture was filtered through celite. The filtrate was washed with water (50 ml) and the organic layer was dried over Na_2SO_4 and stripped under reduced pressure. The product was purified using column chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C)(1:1) as the eluent, to give a colourless oil; b.p89°C under water pump pressure (lit[20] b.p 62°C at 1.5 Torr); R_f= 0.52(petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 3504, 2989, 2939, 2918, 1641, 1481, 1447, 1396, 1373, 1344, 1268(P=O), 1166, 1103, 1055(P-O-R), 962, 784, 715(P-C(H)); $\delta_H(CDCl_3)$ 1.38(t, $C\underline{H}_3CH_2OP$, J= 7Hz), 4.3(m, $CH_3C\underline{H}_2OP$, J= 7.14Hz), 5.97(td, $F_2C\underline{H}$, $J_{H,P}$= 26.9Hz, $J_{H,F}$= 48.7Hz); $\delta_C(CDCl_3)$ 16.3(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 4.4Hz), 64.5(d, $CH_3\underline{C}H_2OP$, J_{CP}= 6.7Hz), 111.8(td, $\underline{C}F_2$, $J_{C,F}$= 259.5Hz, $J_{C,P}$= 213.3Hz).

[3,3-Difluoro-3-(dihydroxyphosphinyl)-2-hydroxy-2-methyl] propionic acid (86)

The methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-hydroxy-2-methyl] propionate (84) (0.1006g, 0.3466mmol) was stirred with TMSI in excess(0.30ml) without solvent at room temperature for 2 days, then heated up to 60°C for 5 days. The excess silylating reagent and ethyl iodide were removed URP to gave the trisilylated

ester. This was dissolved in 50ml of ether and hydrolysed with water (3x20ml), to give a viscous brown product in 100% yield; $\nu_{max}(D_2O)/cm^{-1}$ 3416, 2518, 1724(C=O), 1451, 1209(P=O), 1084; $\delta_H(D_2O)$ 1.44(s, $C\underline{H}_3$); $\delta_C(D_2O)$ 19.0(s, $\underline{C}H_3$), 118.4(td, $\underline{C}F_2$, $J_{C,F}$= 269.9Hz, $J_{C,P}$= 191.4Hz), 173.8(s, C=O); m/z(+ve FAB) 221(MH^+, 100%), 175(M^+-CO_2H, 65), 149(20), 91(60); m/z(-ve FAB) 219(MH^-, 218.9861 $C_4H_7F_2O_6P$ requires 218.9870, 100%).

Diethyl bromodifluoromethanephosphonate (38)

Dibromodifluoromethane (33.2g; 0.2mol) was added to a stirred solution of triethyl phosphite (42.0g, 0.2mol) at room temperature in 15ml of dry diethyl ether. Under nitrogen atmosphere the colourless solution was left stirring at room temperature until the reaction started. Just after the reaction was initiated, the solution was cooled in an ice bath in order to avoid high tempereratures inside the reaction mixture due to the exothermic reaction. Then the mixture was concentrated under reduced pressure, and the product was purified using column chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80 °C) (3:7) as the eluent, to give a colourless oil; R_f= 0.81(petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2988, 1478, 1445, 1394, 1372, 1284, 1284(P=O), 1144, 1091, 1020(OCH_3), 878, 795, 745, 626, 550(C-Br); $\delta_H(CDCl_3)$ 1.42(t, $C\underline{H}_3CH_2OP$, $J_{H,H}$= 7.05Hz), 4.37(m, $CH_3\underline{C}H_2OP$, $J_{H,H}$= 367Hz); $\delta_C(CDCl_3)$ 16.2(d, $\underline{C}H_3CH_2OP$, $J_{C,F}$= 7Hz), 66.2(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 6.6Hz), 119.1(td, $\underline{C}F_2$, $J_{C,F}$= 328.3Hz, $J_{C,P}$= 238.0Hz); m/z(E.I.)267(M^+, 2), 187(M^+-Br, 9), 137(85), 109(100); m/z(C.I.) 267(MH^+, 100%). Anal. Calcd. for $C_5H_{10}BrF_2O_3$: C, 22.5; H, 3.8; Found: C, 22.6; H, 3.9.

[(Diethoxyphosphinyl) difluoromethyl] zinc bromide (39)

Diethyl bromodifluoromethanephosphonate (38)(5.58mmol, 1.85g) was added slowly, at room temperature, to a stirred solution of acid-washed zinc powder (5.58mmol, 0.365g) in 3ml of dry THF. After being stirred for 4 days at room temperature, the solution was filtered to remove any excess of zinc powder, and the solvent was evaporated under reduced pressure to give a colorless viscous compound in 98% yield. ν_{max}(liquid film)/cm^{-1} 3347, 2984, 1622, 1477, 1444, 1395, 1370, 1291(P=O), 1198, 1028(P-O-R), 965, 869, 796, 744, 611, 529; $\delta_H(CDCl_3)$ 1.39 (t, $C\underline{H_3}CH_2OP$, $J_{H,H}$= 7.1Hz), 4.32(m, $CH_3C\underline{H_2}OP$).

Methyl 2-oxo-3,3-difluoro-3-(diethoxyphosphinyl) propionate (98)

To the solution of [(diethoxyphosphinyl) difluoromethyl] zinc bromide (prepared from diethyl bromodifluoromethanephosphonate and acid-washed zinc powder) (5.2mmol, 1.72g) in 3 ml of dry THF, was added a catalytic amount of cuprous bromide(0.1g). Methyl oxalyl chloride (5.2mmol, 0.48ml) was added dropwise, at room temperature, to the reaction mixture and left stirring overnight. The mixture was filtered and poured into 10ml of water and extracted three times with 10ml of ether. The organic layer was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The product was purified using column chromatography on silica gel with ethyl acetate - light petroleum (b.p. 60-80°C)(6:4) as the eluent to give the product in 52% yield; ν_{max}(liquid film)/cm^{-1} 2959, 1745(C=O), 1443, 1394, 1371, 1252, 1205(P=O), 1165, 1024(OCH_3), 974; $\delta_H(CDCl_3)$ 1.39(t, $C\underline{H_3}CH_2O$, $J_{H,H}$= 7.05Hz), 3.93(s, $OC\underline{H_3}$), 4.34(m, $CH_3C\underline{H_2}O$); $\delta_C(CDCl_3)$ 16.1(d, $\underline{C}H_3CH_2O$, $J_{C,P}$= 4.4Hz), 54.0(s, OCH_3), 65.7(d, $CH_3\underline{C}H_2O$, $J_{C,P}$= 6.6Hz), 92.8(q, $-CF_2\underline{C}(O)CO_2Me$, $J_{C,F}$= 13.2Hz) 167.6(dt, CF_2, $J_{C,F}$= 275.4, $J_{C,P}$= 200.5Hz), 167.7(s, C=O); m/z(C.I.) 275(MH^+, 100%), 247(5), 219(2), 73(8).

Methyl [3,3-difluoroethyl-3-(diethoxyphosphinyl)-2-Trifluoromethane sulfonate-2-methyl] propionate (96)

A solution of pyridine (0.35ml) in CH_2Cl_2(10ml) was treated with trifluoromethanesulfonic anhydride (4.22mmol; 0.71ml) at -23°C (CCl_4/dry ice) under slight N_2 pressure with stirring. Then methyl [3,3-difluoroethyl-3-(diethoxy phosphinyl)-2-hydroxy-2-methyl] propionate (**84**)(2.81mmol; 0.82g), dissolved in 5ml of CH_2Cl_2, was added, and the solution was stirred at r.t. for 12h. It was poured into CH_2Cl_2 (20ml), washed with HCl 2M (2x5ml) and with distilled water(2x5ml). The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. Column chromatography on silica gel, with ethyl acetate-light petroleum (b.p. 60-80°C) (1:1) as the eluent, yielded the title compound as a pale oil (0.37g, 31%); R_f= 0.82 (petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2989, 2360, 1768(C=O), 1419(C-OSO_2CF_3), 1282, 1214(P-O-R), 1019; δ_H($CDCl_3$) 1.38(t, $C\underline{H}_3CH_2OP$, $J_{H,H}$= 7.15Hz), 2.08(s, $C\underline{H}_3$), 3.92(s, $OC\underline{H}_3$), 4.28(m, $CH_3C\underline{H}_2OP$,); δ_C($CDCl_3$) 16.3(d, $\underline{C}H_3CH_2OP$, $J_{C,P}$= 4.4Hz), 16.8(s, C-$\underline{C}H_3$), 54.4(s, OCH_3), 65.6(d, $CH_3\underline{C}H_2OP$, $J_{C,P}$= 6.6Hz), 117.9(td, CF_2, $J_{C,F}$= 319.5Hz, $J_{C,P}$= 241.2Hz); m/z(+ve FAB) 423(MH^+, 100%), 273(M^+- SO_3CF_3, 5)

Attempted preparation of Methyl 2-[(diethoxyphosphinyl)difluromethyl] propeonate (86)

Method A

The reaction was done in the kygelrohr. Methyl [3,3-difluoro-3-(diethoxy phosphinyl)-2-hydroxy-2-methyl] propionate (**84**)(55 mg) was placed with $CUSO_4$ (dried), and it was left reacting for 6h at 120°C. Then, it was distilled under vacuum at temperature from 120°C to 200°C. The IR and NMR showed only the starting material.

Method B

A solution of methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-hydroxy-2-methyl] propionate (**84**)(0.36mmol) in dry toluene(10 ml) was placed together with iodine in a 25 ml round bottomed flask. The mixture was stirred at 130°C for 6h, and followed by TLC each hour. Unfortunately, the desired olefin was not formed.

Method C

1- Methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-trifluoromethane sulfonate-2-methyl] propionate (**96**) was dissolved in 5ml of CH_2Cl_2 and then added to a stirred solution of LDA, DBU, BuLi in CH_2Cl_2(5ml) at 0°C. It was warmed to RT and letft stirring for 17h. The reaction was followed by TLC and it showed the decomposition of compound (**96**).

2- Methyl [3,3-difluoro-3-(diethoxyphosphinyl)-2-trifluoromethane sulfonate-2-methyl] propionate (**96**) was treated with 5ml of dry pyridine under N_2 at 0°C, and then warmed to RT. Since the reaction did not proceed, it was heated up to 50°C. The product was poured into ethylacetate and washed with HCl 2M (2x5ml) and H_2O (1x5ml), and the organic layer was dried over Na_2SO_4 and concentrated URP. Unfortunately, the desired compound was not formed.

Method D - Wittig Reaction

1- To the solution of methyl 2-oxo-3,3-difluoro-3-(diethoxyphosphinyl) propionate (**98**) in dry toluene (6ml), at -90°C with an Et_2O/N_2 cooling bath, Tebbe Reagent (6ml) was added dropwise. Then the reaction was allowed to warm to 0°C and poured into a vigorously stirred two-phase mixture of CH_2Cl_2 and satured aqueous $NaHCO_3$ to afford an emulsion. After filtering through celite to remove the titanium and aluminium salts, the phases were separated and the aqueous phase was extracted with CH_2Cl_2. The reaction mixture was analyzed by TLC (silica gel, petrol-ethyl acetate 1:1) using

ammonium molibdade. Unfortunately, the desired olefin was not detected.

2- To a suspension of zinc dust (1.2g) and diiodomethane (1.66g, 6.2mmol) in dry THF (6ml) was added a hexane solution of trimethylaluminium (0.62ml; 1.2mmol) at 25°C. The resulting mixture was stirred for 10 min. A solution of methyl 2-oxo-3,3-difluoro-3-(diethoxyphosphinyl) propionate (98)(0.85g, 3.1g) in THF was added dropwise at O°C. After stirring at this temperature for 4h, the reaction mixture was diluted with ether (10ml), poured into 1M hydrochloric acid (20ml), and extracted with ether. The separated organic layer was washed with brine (2x20ml), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was analyzed by TLC (silica gel, petrol-ethyl acetate 1:1) using ammonium molybdate. Unfortunately, the desired olefin was not detected.

3- To a stirred -78°C suspension of methyltriphenylphosphonium bromide (0.78g, 2.2mmol) in dry THF (3ml) under nitrogen was added dropwise *n*-BuLi (1.4ml). The mixture was warmed to room temperature for 30 min. The solution was then cooled to -78°C and added slowly to a stirred -78°C solution of methyl 2-oxo-3,3-difluoro-3-(diethoxyphosphinyl) propionate (98)(0.3g, 1.1mmol) in THF (3ml). The mixture was then warmed to room temperature for 3h, quenched with water (10ml), and extracted with ethyl acetate. Unfortunately, the desired olefin was not formed.

PART II

OXAZIRIDINE COMPOUNDS

CHAPTER FOUR

INTRODUCTION

PART II - OXAZIRIDINE COMPOUNDS

CHAPTER FOUR - INTRODUCTION

4.1 Introduction

Three-membered rings with one hetero atom have been known since the 19th century. Nowadays, they are of great interest both scientifically and technically. A three-membered ring with two hetero atoms can also be formed, and in the last few years many such rings have been synthesized.

The first family which proved to be a true three-membered ring containing two hetero atoms were the oxaziridines, prepared in 1952 by H. Krimm[84]. The compounds were originally named isonitrones, because they are isomeric with the nitrones or oxime-N-ethers. Subsequently they were called oxaziranes. However, the oxaziridine nomenclature would appear to be more descriptive, and recently this name has gained general acceptance[84,85] for these compounds.

The numbering of the oxaziridine system, and the structure of nitrones are shown in **Figure 29.**

Structure of oxaziridines Structure of nitrones

Figure 29 - Structures of oxaziridines and nitrones

Oxaziridines are unique three-membered heterocyclic compounds constructed of three kinds of atoms having different electronegativities in adjacent positions[86]. The oxaziridines have a ring system similar to that of aziridine, except for a stronger

electronegative oxygen and a much more weak basic nitrogen lone pair.

Most oxaziridines are liquids with boiling points somewhat above that of the corresponding imine from which they are derived. They have, however, much lower boiling points than the isomeric highly polar nitrones. The oxaziridines which are solids have substantially lower melting points than the corresponding nitrones. In general, oxaziridines have limited water solubility, again in contrast with nitrones. They have a characteristic unpleasant smell and are non-basic.

Extensive investigations of oxaziridines have revealed their unusual reactivity, undoubtedly related to the presence of the strained three-membered ring and a relatively weak N- O bond[87]. The consequence of these features is the low basicity of the oxaziridine nitrogen compared to amines. Another remarkable property of some oxaziridines is that they possess a configurationally stable nitrogen atom at ordinary temperatures. For the N-alkyl oxaziridines the experimentally determined inversion barriers are in the range of 24 to 31 Kcal mol^{-1}[88].

The oxaziridines show in their IR spectra a well developed band between 1430 and 1470 cm^{-1}, which is assumed to be due to C-H bending, and has been considered characteristic for oxaziridines[85,89].

An important point concerning an unsymmetrically substituted oxaziridine ring is that it has an asymmetric carbon and should be capable of resolution into optically active forms.

Oxaziridines have recently received attention as potential anti-tumor agents and as analogues of penicillin[90]. In the search for new anticancer agents involving small ring systems, Sosnovsky *et al.*[91] also focused on certain derivatives of oxaziridine systems.

The most important clinical problems encountered in anti-biotherapy using β-lactam derivatives, arise from the widespread emergence of resistant pathogenic bacteria producing β-lactamases[92]. Some efforts have been made to discover new active β-lactams that will overcome the bacterial defense mechanism. J.

Marchand-Brynaert *et al.*[92] have examined several classes of heterocycles able to interact with the penicillins binding proteins (PBPs) in the same manner as the β-lactams, and they considered oxaziridines as potential irreversible alkylating inhibitors of the bacterial serine D,D-transpeptidases (**Figure 30**).

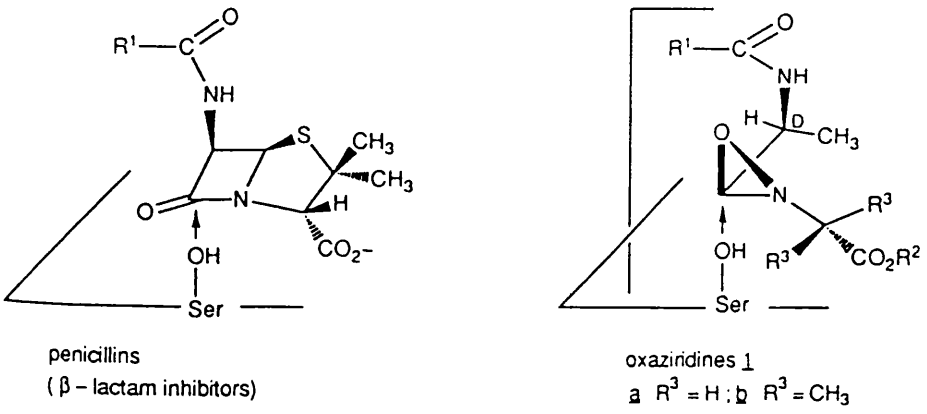


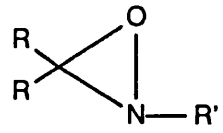
Figure 30

It has been shown that some compounds having a small heterocyclic ring, such as derivatives of oxirane and aziridine, are active as bioalkylating agents and some of them, *e.g.* the antibiotic mitomycin C, or the synthetic drug tri(1-aziridinyl)-*p*-benzoquinone (Trenimon), are clinically used in cancer chemotherapy. It was also postulated that (2-aziridinyl)- and oxiranyl-*p*-quinones should be effective bioalkylators and thus could be potent cytostatic agents[93]. Some other oxaziridines are extensively studied as substrates[94], or as oxygen-transfer agents[88].

Mlochowski *et al.*[95] synthesized a novel group of oxaziridines having the small heterocyclic ring conjugated with *p*-quinone, and their chemical properties and activities against experimental tumor cells were established and discussed.

Recently, Hata and Watanabe[96] studied the biological properties of oxaziridines and found strong cytotoxic activity in their derivative.

Stable oxaziridines (**Figure 31**) are capable of oxidizing I^- to I_2 and phosphines to phosphine oxides. However, they are not sufficiently reactive to oxidize sulfides to sulfoxides or to epoxidize alkenes[93].



(R' = alkyl, aryl, H)

Figure 31 - Stable oxaziridines

Davis *et al*[93] have shown that 2-sulfonyloxaziridines are aprotic, neutral oxidizing reagents that exhibit reactivity similar to peroxy acids, but are more selective. Thus, 2-sulfonyloxazidines have found applications in the oxidation of carbanions to alcohols and phenols, and in the oxidation of enolates. Chiral 2-sulfonyloxaziridines are asymmetric oxidizing reagents for the epoxidation of alkenes, and the oxidation of sulfides to sulfoxides, selenides to selenoxides, and enolates to α-hydroxy-carbonyl compounds (**Figure 32**).

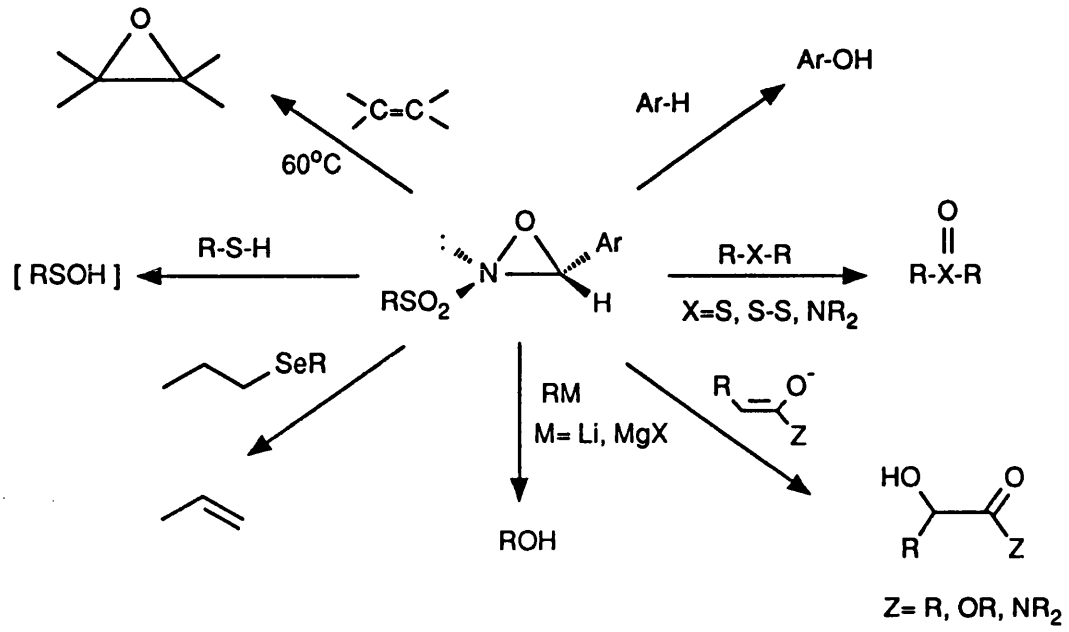
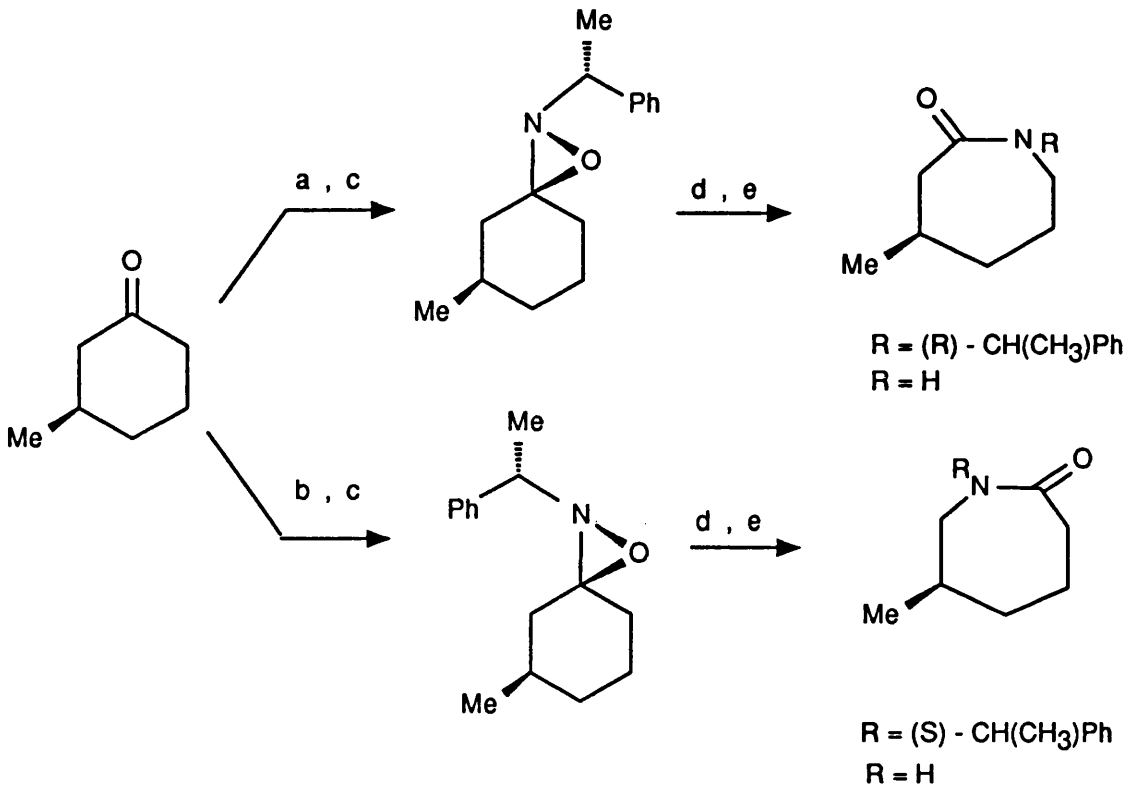


Figure 32 - Oxygen-transfer reactions of 2-sulfonyloxaziridines

Oxaziridines also provide useful alternatives to the Beckmann rearrangement and Schmidt reaction for ring enlargement of cyclic ketones. The conversion of cyclic ketones to heterocycles, using ring-expansion reactions, has been featured in the preparations of such disparate materials as nylon (cyclohexanone → caprolactam) and the plant growth promoter brassenolide (Baeyer-Villiger reaction on a 6-keto steroid)[97]. J. Aube *et al.*[97] prepared some spirocyclic oxaziridines from unsymmetrical ketones. The ring expansion for the oxaziridine resulting from the reaction of 3-methyl cyclohexanone with (R)-α-methylbenzylamine (α-MBA), followed by oxidation with (+)-monoperoxycamphoric acid ((+)-MPCA), is shown in **Figure 33.**



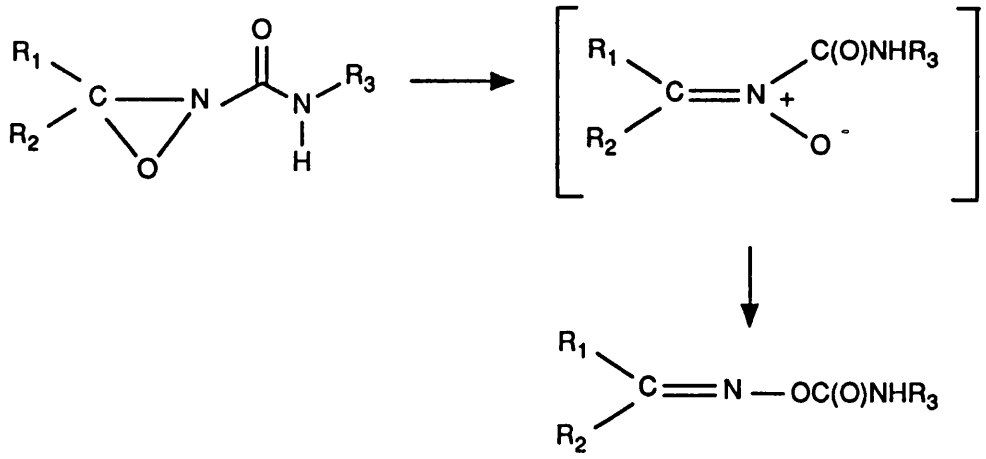
Reagents : (a) (R) - α - MBA ; (b) - (S) - α - MBA ; (c) (+) - MPCA ; (d) hν , 254 nm , quartz tube ; (e) Na / NH_3

Figure 33 - Ring expansion for the oxaziridine derived from 3-methylcyclohexanone

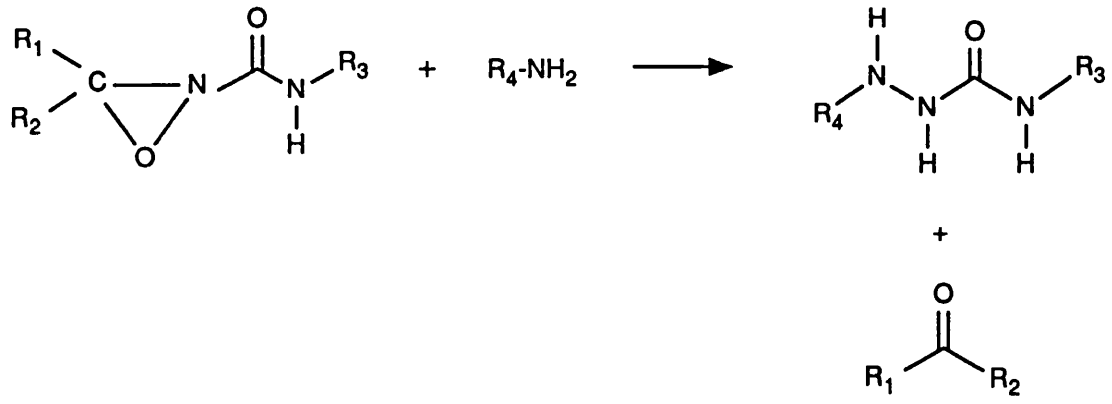
Since oxaziridines are three-membered ring systems composed of carbon, nitrogen, and oxygen atoms, it can be envisaged that bond rupture between the carbon and the hetero atoms, as well as between the hetero atoms themselves, can result in a variety of products. Ring opening of the strained oxaziridine three-membered ring is the key to all of the synthetically useful reactions of oxaziridines.

Some of the important transformations of the oxaziridine ring involving such bond cleavages are:

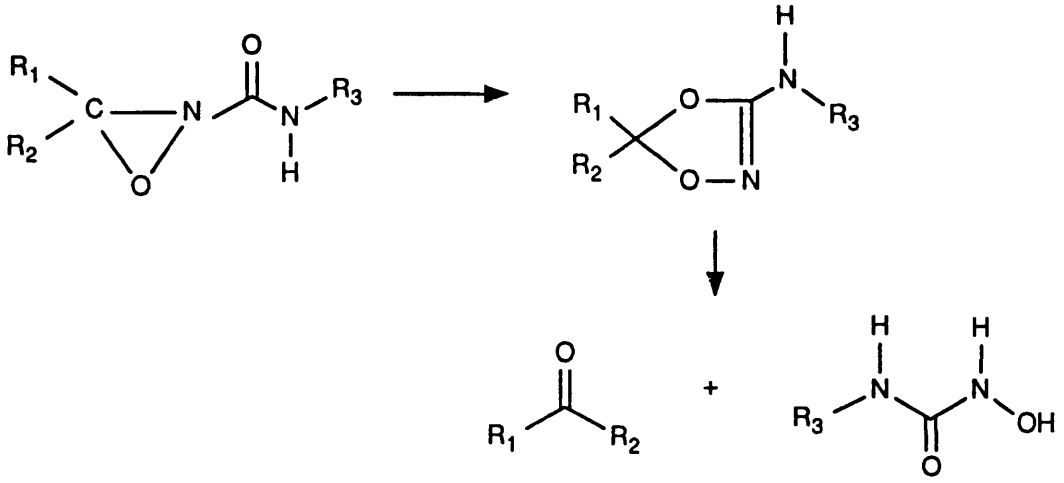
a) Rearrangement of certain oxaziridines to oximes via a nitrone intermediate[91].



b) The amination reaction resulting in the formation of a semicarbazide derivative[87].

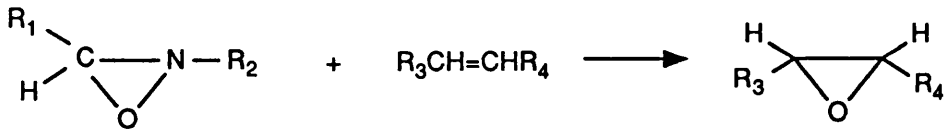


c) The rearrangement of certain oxaziridine derivatives via 1,3,4-diazoles to give hydroxyurea derivatives (**99**)[98].



(99)

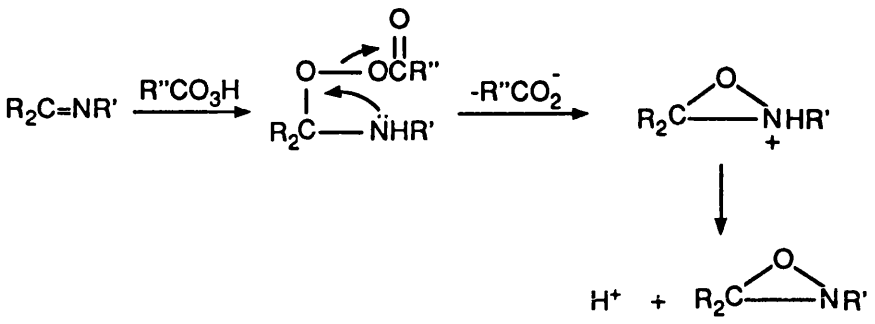
d) The epoxidation of unsaturated compounds by oxaziridines could probably lead also *in vivo* to alkylating species which could interact with the DNA[87].



Some other interesting reactions of oxaziridines are described in section 4.3.

4.2 Synthesis of Oxaziridines

The most useful route for the preparation of oxaziridine is the oxidation of imines with organic peracids[99]. This is a remarkably general reaction and, since imines of widely varying structure can easily be prepared, this oxaziridine synthesis is a versatile one. It is possible that the imine reaction proceeds through addition of the peracid to the imine, followed by internal nucleophilic displacement of the basic nitrogen atom (**Scheme 57**).



Scheme 57

The major limitation of the synthesis is the extremely labile nature of some oxaziridines.

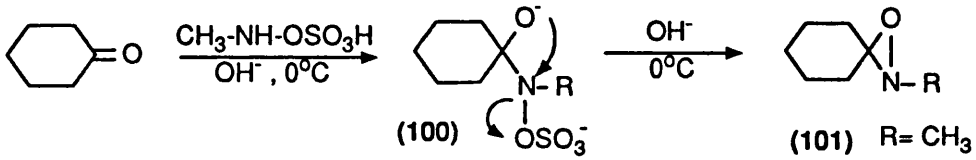
The process can be simplified. It is not always necessary to use a pre-formed Schiff's base. Often, it is sufficient to bring the carbonyl compound and the amine together in an inert solvent and to add the peracid to the mixture later. In this way oxaziridines can be obtained in good yield, even if the Schiff's base is unknown, or can be only obtained in poor yield.

Optically active oxaziridines have been prepared by the oxidation of achiral imines with chiral peracids[100], by thermal isomerization in a chiral liquid crystal[101], and by photochemical synthesis in a chiral solvent[102].

Emmons[94,99] prepared oxaziridines by addition, at 10-20°C, of an essentially anhydrous solution of peracetic acid in methylene chloride to the imine dissolved in the same solvent. The peracetic acid was prepared at ice-bath temperatures by reaction of acetic anhydride with 90% hydrogen peroxide, in the presence of a catalytic amount of sulfuric acid. The reagent was then diluted with methylene chloride and allowed to react with the azomethine under appropriate experimental conditions. Yields of the oxaziridines generally were of the order of 50-80%, depending on the properties and stability of the particular compound obtained.

According to E. Schmitz[84,89], oxaziridines can be obtained when

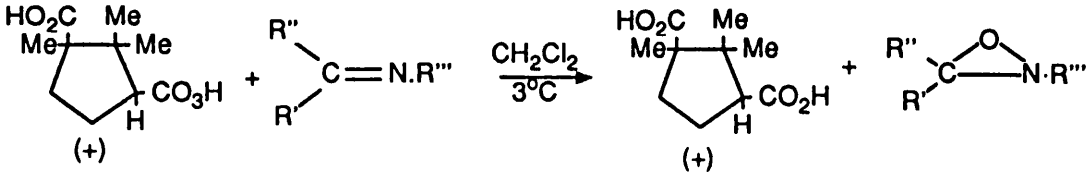
methylhydroxylamine-O-sulfonic acid in dilute sodium hydroxide is allowed to act on cyclohexanone. A strongly oxidizing, ether-soluble substance is formed within a few minutes (**Scheme 58**). The properties of the obtained compound are analogous to those of oxaziridines prepared by other known methods[94]. He also pointed out that oxaziridines are formed from other carbonyl compounds, e.g. benzaldehyde. However, the reaction rapidly reaches a limit with the increasing size of the N-alkyl residue; with cyclohexanone, the yield falls from 60% for the N-methyl compound (**101**) to 10% for the N-ethyl compound. No oxaziridine is formed with N-t-butyl-hydroxylamine-O-sulfonic acid.



Scheme 58

The rear-side attack of the alkoxide oxygen in (**100**) is sterically hindered as the size of the N-alkyl residue increases. Thus when R= t-butyl, the leaving group is in the particularly strongly sterically hindered neopentyl position.

D. R. Boyd[103] synthesized optically active oxaziridines, using a method that involves the reaction between imines and peracetic or m-chloroperbenzoic acid.

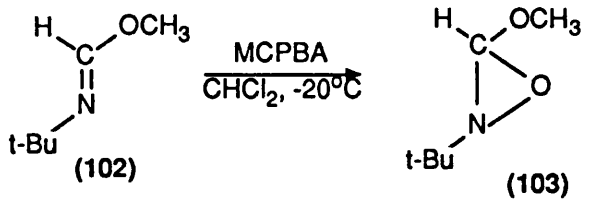


imine	oxaziridine [α_D]
PhCH=NBu^t	-12.7°
α-C_6H_5CH=NBu^t	-4.3°
Pr^{i}CH=NBu^t	- 3.3°
(Bu^i)MeCH=NPr	-9.0°
p-$NO_2C_6H_4$CH=NPr^i	-12.0°

Scheme 59

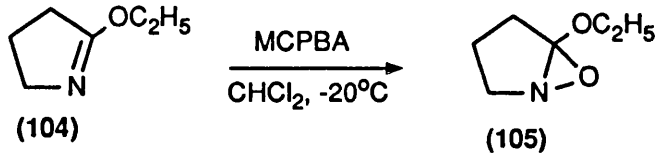
When optically active peracids are used to oxidize certain sulphides and olefins, chiral sulphoxides and epoxides are formed. Imines have now been shown to give optically active products on treatment with (1S)-(+)-percamphoric acid at 3°C, in dichloromethane solution (**Scheme 59**). Yields in the imine -(+)-percamphoric acid reactions are generally good (47 to 80%).

Thomas *et al.*[104] reported the peracid oxidation of some strained and unstrained imino ethers to alkoxyoxaziridines. Reaction of O-methyl-N-t-butyl formimidate (**102**) with meta-chloroperbenzoic acid (MCPBA) in dichloromethane at -20°C gives 2-t-butyl-3-methoxyoxaziridine (**103**) in 86% yield (**Scheme 60**).



Scheme 60

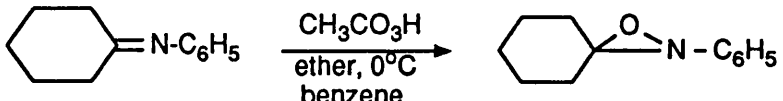
The reaction of 1-aza-2-ethoxycyclopentene (**104**) with MCPBA gave 5-ethoxy-1-aza-6-oxabicyclo-[3.1.0] hexane (**105**) in 66% yield (**Scheme 61**).



(104) (105)

Scheme 61

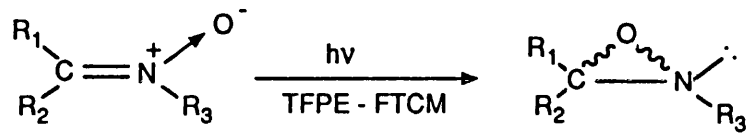
Meyer *et al.*[105] synthesized the spirooxaziridine (**107**) by addition of the anil (**106**) in anhydrous ether to an anhydrous solution of peracetic acid in benzene diluted with ether at 0°C. After standing at room temperature for one hour the reaction mixture was washed in turn with 10% sulfuric acid and 10% sodium carbonate, and finally dried over potassium carbonate. The solvents were then removed by distillation under reduced pressure, and the residue dissolved in petroleum ether. On filtration of this solution through celite and activated charcoal, and subsequent concentration and cooling, compound (**107**) crystallized out (**Scheme 62**).



(106) (107)

Scheme 62

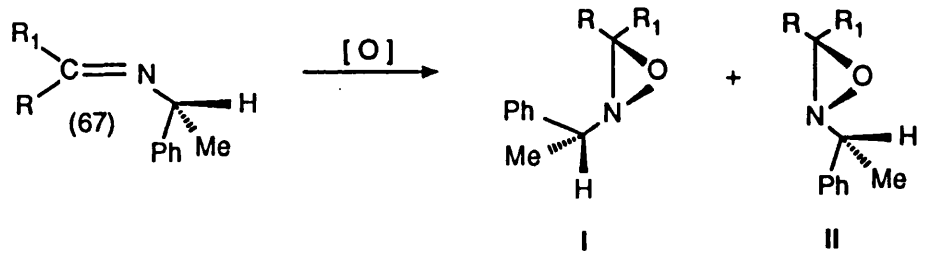
Boyd *et al.*[102] synthesized optically active oxaziridines by photochemical rearrangement of isomeric nitrones in (+)- or (-)-2,2,2-trifluoro-1-phenylethanol-fluorotrichloromethane solvent (**Scheme 63**).



R_1	R_2	R_3	$[\alpha_D]$	Optical purity
Ph	Ph	Bu^t	+75.9	29
Ph	Ph	Pr^i	+38.8	20
$C_6H_4NO_2$-p	H	Bu^t	-5.6	6

Scheme 63

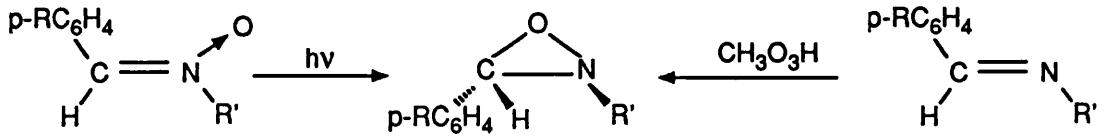
Belzecki *et al.*[88] studied the asymmetric synthesis of oxaziridines. They referred to the formation of nonracemic diastereomeric 3,3-disubstituted oxaziridines in a high yield, by the oxidation of the Shiff's bases that are formed by the reaction of chiral (R)-(+)-α-phenylethylamine and a carbonyl compound using m-chloroperbenzoic acid (**Scheme 64**).



Starting (67)	% diastereoisomers I	II
Me, Me, C=	82	!8
C=	87	13
C=	97	3

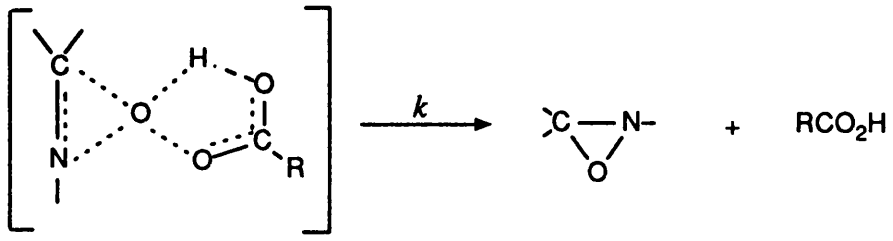
Scheme 64

Splitter *et al.*[106] synthesized oxaziridines by the irradiation of N,α-diarylnitrones. Some of these oxaziridines were shown to be identical with those synthesized by the peracetic acid method[94] (**Scheme 65**).



Scheme 65

Bucciarelli *et al.*[107], postulated the following mechanism for the imine-peroxyacid reaction: a concerted electrophilic attack of the peroxy acid on the electrons of the C=N bond with a symmetric cyclic transition state.



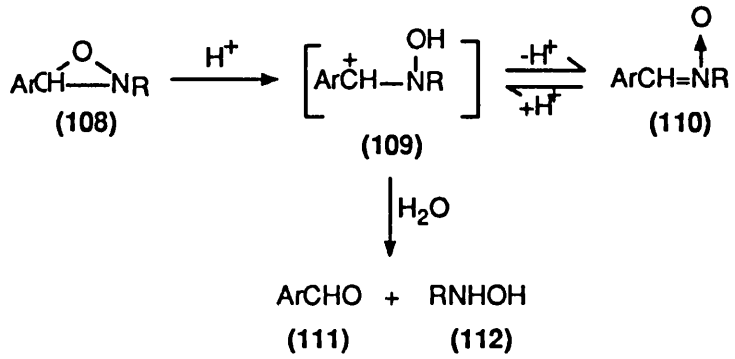
4.3 Reactions of Oxaziridines

The oxaziridines display their high-energy in reactions involving the opening of the three-membered ring, whether they involve reducing agents, acids, alkalis, radical reagents, or oxidizing agents. The opening of the strained oxaziridine three-membered ring is the key to all the synthetically useful reactions of oxaziridines. An unusual property of oxaziridines is their ability to react as both aminating and oxygenating reagents with nucleophiles. The site of nucleophilic attack on the oxaziridine three-membered ring is determined by the substitution pattern at nitrogen. Concerning this property, Hata *et al*[96] demonstrated that oxaziridines act as aminating reagents when the groups attached to the oxaziridine nitrogen (**Figure 29**) are small (i.e. R_1= H, Me). As R_1 becomes larger, the site of attack is shifted from nitrogen to oxygen. In contrast to oxiranes and aziridines, nucleophiles generally do not react at the oxaziridine carbon atom.

4.3.1 Reaction with Acidic Reagents

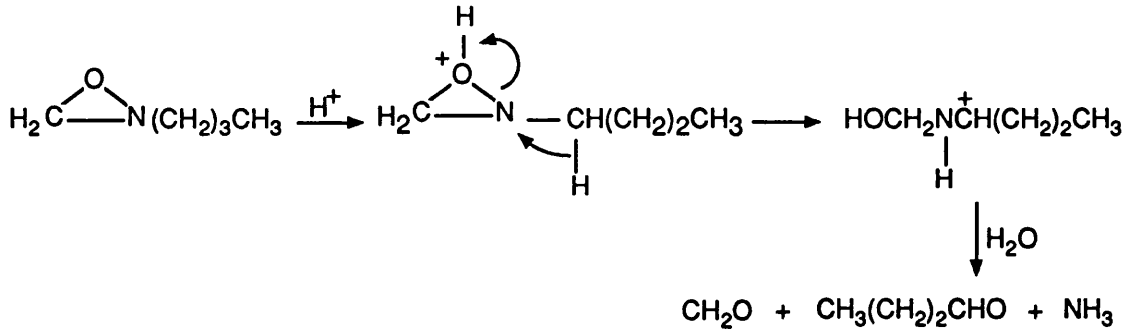
The hydrolysis of 3-aryloxaziridines (**108**) takes place readily in the presence of a strong mineral acid. Thus 2-*tert*-butyl-3-phenyloxaziridine is converted by sulfuric acid in aqueous methanol into benzaldehyde and *tert*-butylhydroxylamine in quantitative yield. It is probable that, to some extent, the nitrone is involved as an intermediate in this reaction. The unstable intermediate (**109**) is converted into nitrone

(110) by loss of a proton, and by reaction with water the hydrolysis products (111 and 112) are obtained[99] (Scheme 66).



Scheme 66

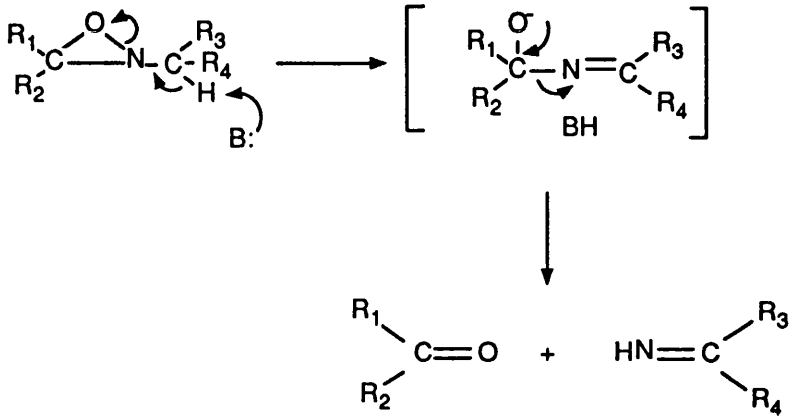
The reaction of alkyloxaziridines with aqueous acid follows a different course[99]. In this case the oxygen-nitrogen bond of the protonated oxaziridine is cleaved via a concerted reaction, with concurrent migration of one of the groups on the α-carbon atom of the N-alkyl substituent. In the transition state for this reaction the nitrogen atom is electron-deficient, and this provides the driving force for the rearrangement. Thus, the products of this reaction are an aldehyde, or a ketone, and an amine (Scheme 67).



Scheme 67

4.3.2 Reaction with Basic Reagents

The oxaziridine ring itself is generally inert towards bases[88]. However, oxaziridines with a proton on the carbon adjacent to the ring nitrogen undergo a base-promoted fragmentation to aldehydes, or ketones, and imines[108] (**Scheme 68**).

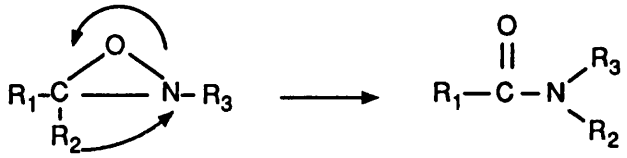


Scheme 68

4.3.3 Pyrolysis and Thermal Decomposition

The products of thermal decomposition vary considerably with the structure of the oxaziridine, and also with the pyrolysis conditions. The thermal decomposition products of 3-aryl oxaziridines are the isomeric nitrones. This is, apparently, a general reaction and may be conveniently accomplished by heating solutions of the oxaziridines in acetonitrile, or diethyl carbitol, at 80-100°C for an appropriate time. This reaction was studied in detail for 2-*tert*-butyl-3-phenyl oxaziridine, and proceeded in quantitative yield[89]. The behavior of various alkyloxaziridines at elevated temperatures under pyrolysis conditions has also been studied. These reactions are carried out in the gas phase at 200-300°C; they are a direct consequence of the

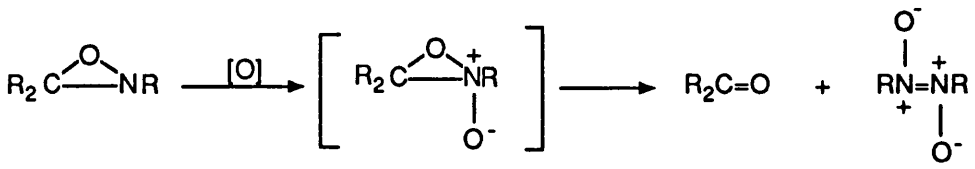
relatively weak oxygen-nitrogen bond and can be described as a concerted rearrangement (Scheme 69).



Scheme 69

4.3.4 Oxidation to Nitrosoalkanes

From a preparative point of view one of the most important oxaziridine reactions is their oxidation to nitrosoalkanes[89]. This is conveniently carried out with peracetic acid at room temperature or below. The nitrosoalkanes are obtained as the trans-dimers which are normally crystalline solids. The reaction can be formulated as in Scheme 70.

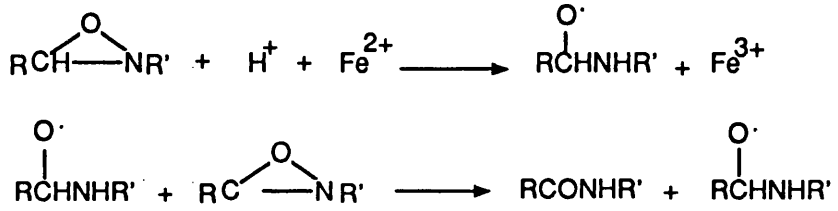


Scheme 70

4.3.5 Reactions with Ferrous Ions

A direct attack on the oxaziridine ring occurs with ferrous salt[94]. Oxaziridines are decomposed by aqueous solutions of ferrous ammonium sulfate even at room temperature ; less than stoichiometric amounts of the ferrous salt cause the

decomposition. The reaction follows a radical-chain mechanism; Emmons[94] proposed as the chain starting reaction a direct attack of the ferrous ion on the oxaziridine ring with the formation of an O-radical. By the attack of a further molecule of oxaziridine, formation of the acid amide occurs with the re-formation of the O-radical which carries on the chain (**Scheme 71**).



Scheme 71

This reaction with bis- or tris-oxaziridine derivatives could possibly result in a cross-linking of the DNA[95].

The objectives of the following section of the thesis were to explore the future of the chemistry of oxaziridines. Firstly, tris-oxaziridines would be sought, and their potential as improved oxidants would be explored. Secondly, if superior efficacy was established, possible catalytic oxidation systems would be investigated.

CHAPTER FIVE

RESULTS AND DISCUSSION

CHAPTER FIVE - RESULTS AND DISCUSSION

5.1 Aims and Objectives

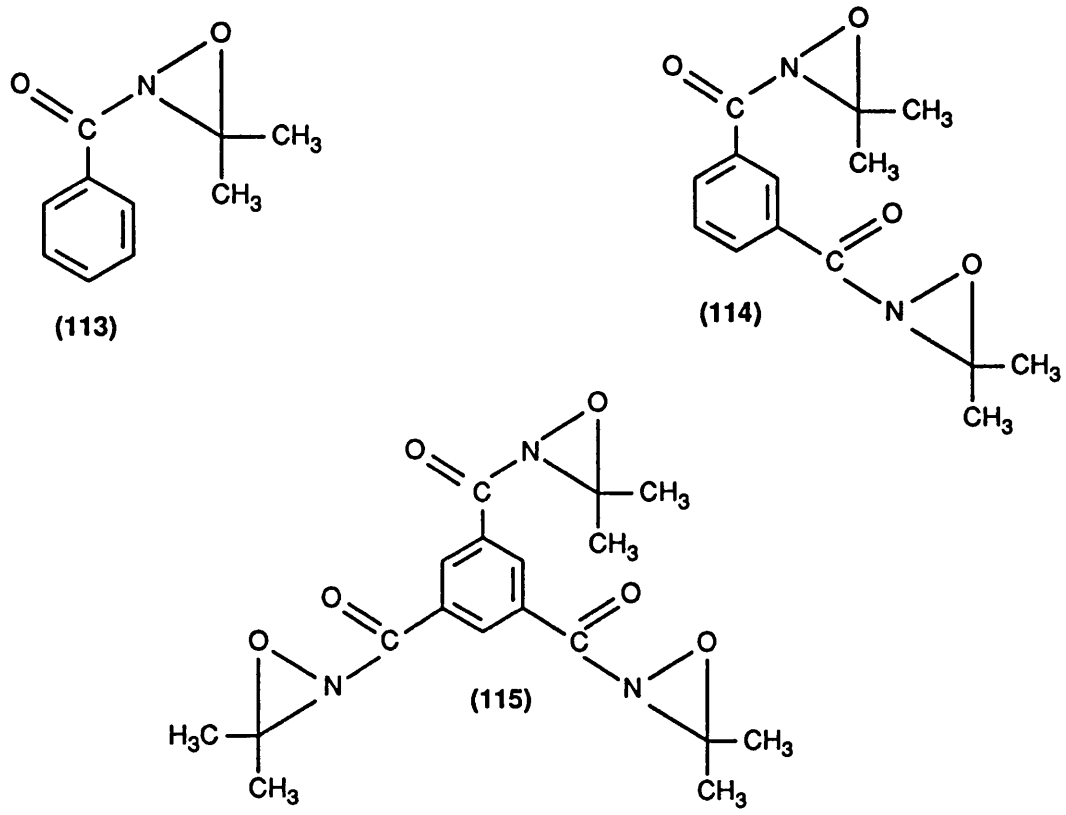
Another objective of this study was the synthesis of some novel stable oxaziridines that could be used as bonding agents in polymerization processes, and also as oxidizing reagents capable of oxidizing sulfides to sulfoxides, selenides to selenoxides, enolates to carbonyl compounds, and alkenes to their corresponding epoxides.

The desired oxaziridine target compounds **(113)**, **(114)** and **(115)** **(Figure 34)**, were all derived from the coupling reaction of 3,3-dimethyloxaziridine with benzoyl chloride, isophthaloyl dichloride, and 1,3,5- benzene tricarbonyl trichloride respectively.

Some attempts to synthesize 3,3-dimethyloxaziridine were made using the classical method described by Emmons[94], *i.e.* the reaction of ammonia with acetone at 0°C to give the corresponding Schiff's base. This Schiff's base would be oxidized with MCPBA at 0°C yielding the 3,3-dimethyloxaziridine. Further reaction with benzoyl chloride, isophthaloyl dichloride, and 1,3,5-benzenetricarbonyl trichloride, would give the corresponding oxaziridine targets, compounds **(113)**, **(114)** and **(115)** **(Scheme 72)**.

Unfortunately, 3,3-dimethyloxaziridine was not isolated, because oxaziridines unsubstituted on the nitrogen atoms are usually unstable[84].

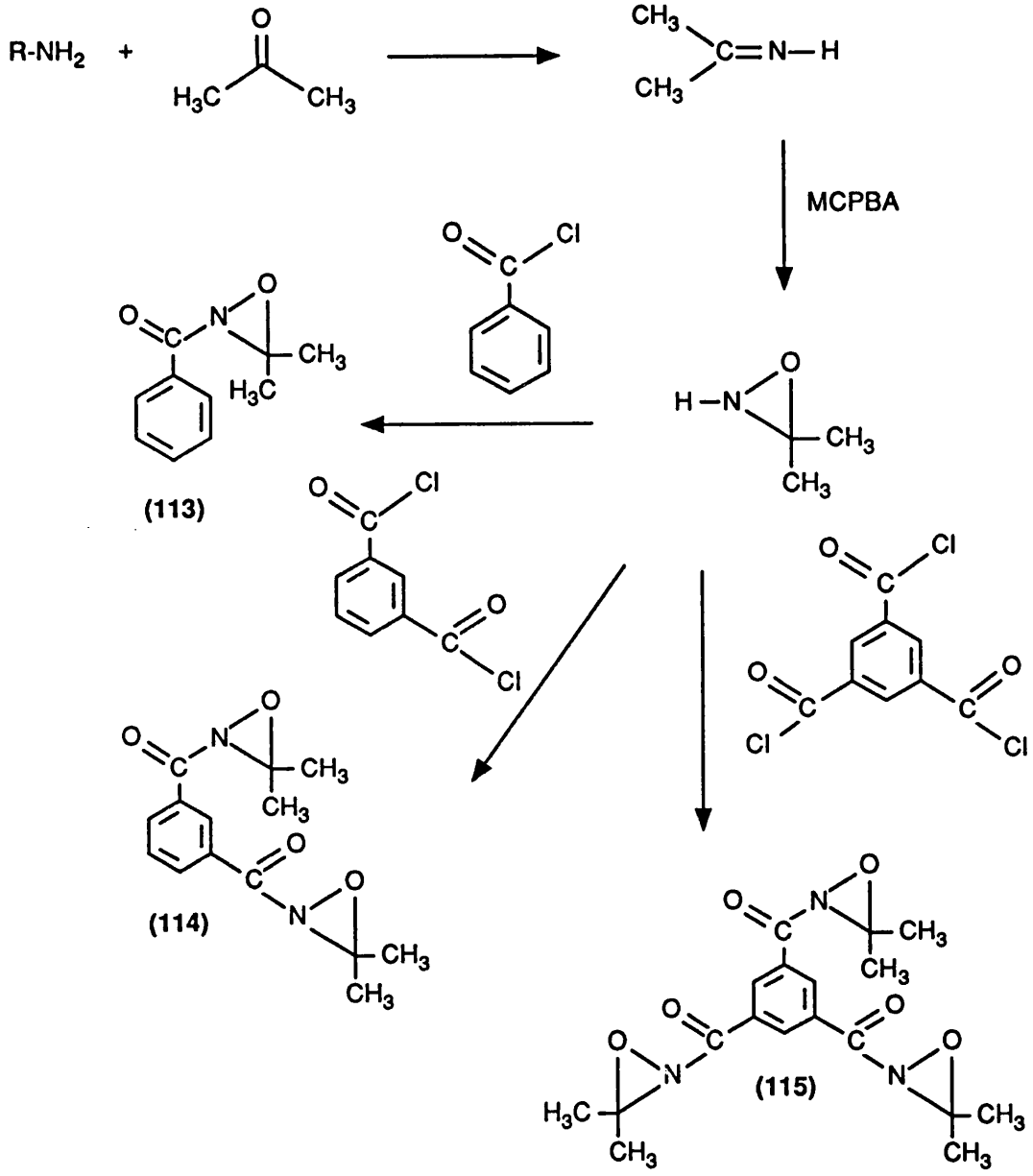
However, the synthesis of compounds **(113)**, **(114)** and **(115)** was possible using the method described by Schmitz[84], *i.e.* reacting hydroxylamine-O-sulfonic acid in dilute sodium hydroxide with acetone using ether, dichloromethane, or benzene as solvent. The resultant 3,3-dimethyloxaziridine was reacted *in situ* with benzoyl chloride, isophthaloyl dichloride, and 1,3,5-benzenetricarbonyl trichloride, yielding the corresponding oxaziridines **(113)**, **(114)** and **(115)** respectively **(Scheme 73)**. The individual syntheses are detailed in the following sections.



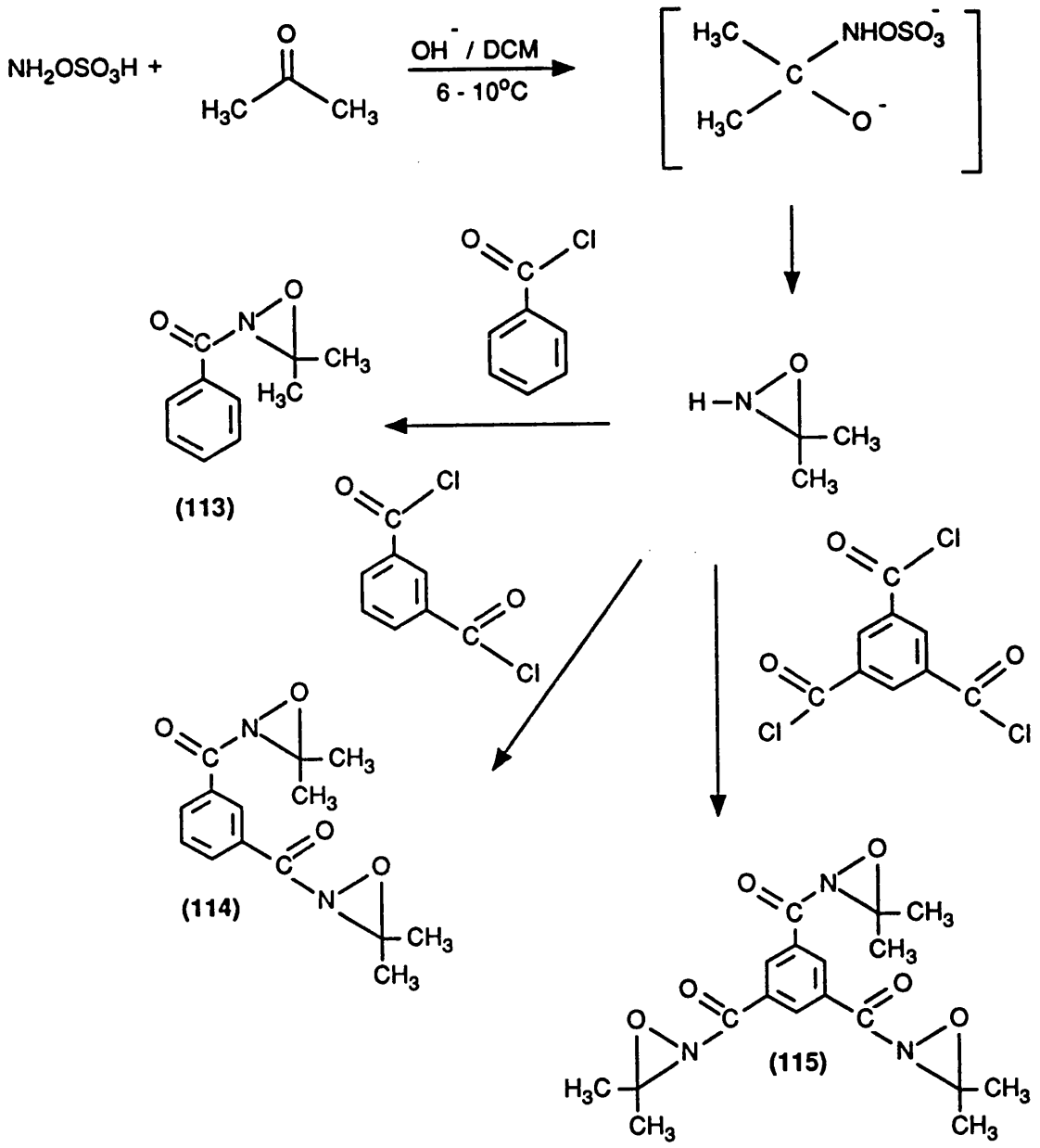
(113) 2-Benzoyl-3,3-dimethyloxaziridine ; **(114)** 1,3-Benzenedicarbonyl-bis (3,3-dimethyloxaziridine); **(115)** 1,3,5-Benzene tricarbonyl-tris (3,3-dimethyloxaziridine)

Figure 34

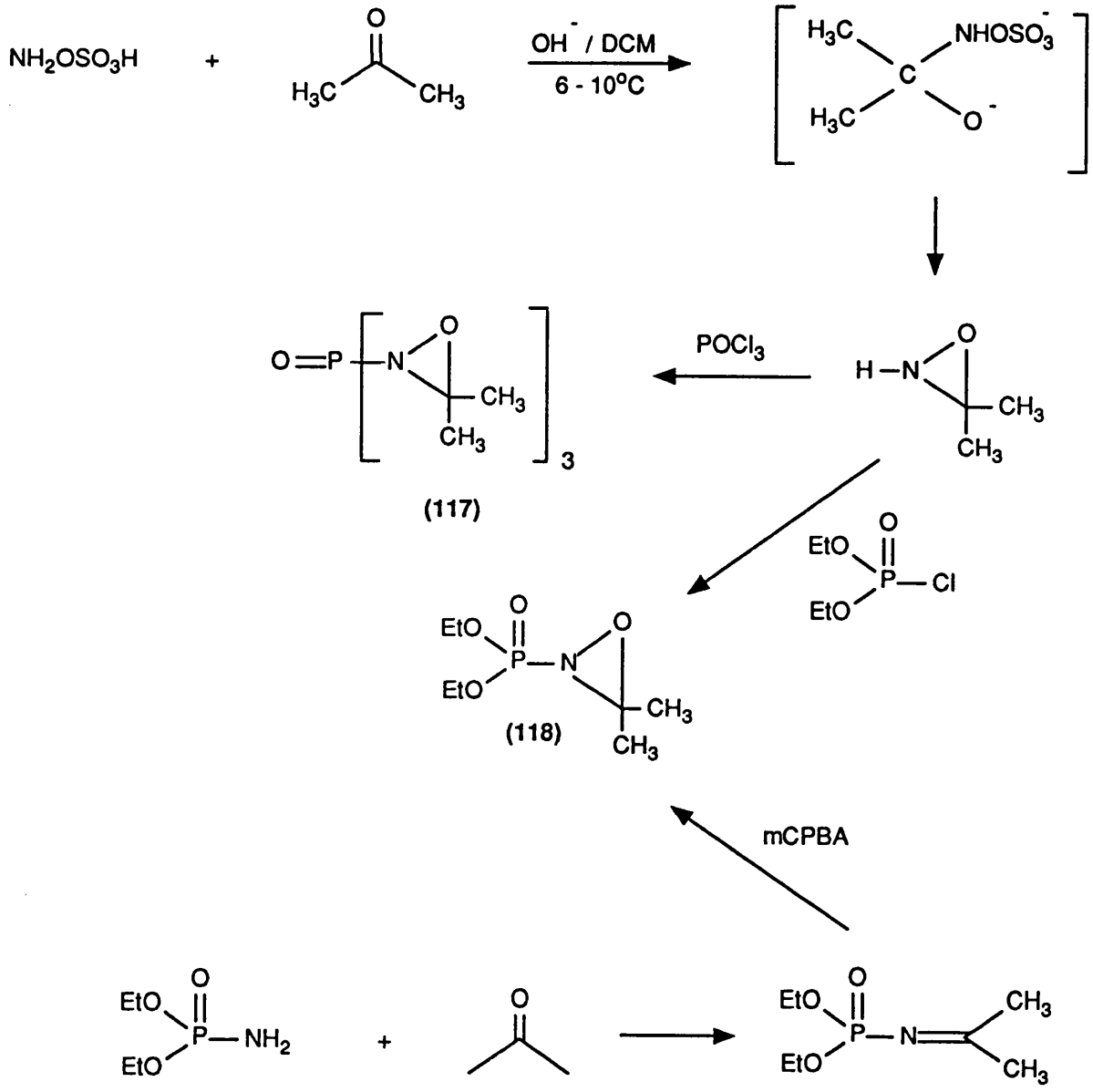
Several attempts to synthesize the tris [3,3-dimethyloxaziridine-2-yl] phosphine oxide (**117**) and 2-diethoxyphosphinyl-3,3-dimethyloxaziridine (**118**) (**Scheme 74**), which could also be tested as a potential oxidising agents, were done. Unfortunately all the attempts failed.



Scheme 72



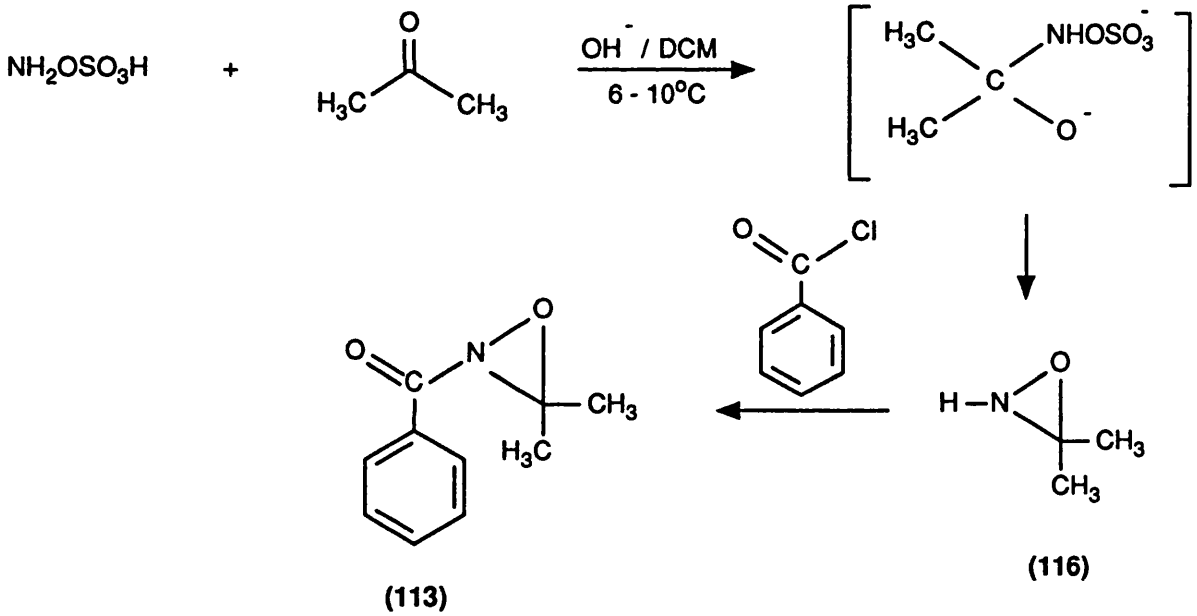
Scheme 73


NH2OSO3H
+
OH- / DCM
6 - 10°C
H3C
NHOSO3-
C
H3C
O-
POCl3
O=P
N
O
CH3
CH3
3
(117)
H-N
EtO
P
Cl
EtO
(118)
mCPBA
NH2
(117)
(118)

Scheme 74

5.2 Synthesis of 2-Benzoyl-3,3-dimethyloxaziridine (113)

2-Benzoyl-3,3-dimethyloxaziridine (**113**) was prepared according to the general procedure of Schmitz *et al.*[98] for the synthesis of oxaziridines. Hydroxylamine-O-sulfonic acid reacted with acetone in a 2N sodium hydroxide solution to form 3,3-dimethyl oxaziridine (**116**). Since this oxaziridine has limited stability, it was reacted *in situ* with benzoyl chloride to give the 3,3-dimethyl-2-benzoyl oxaziridine as a pale oil in 15% yield (**Scheme 75**).



Scheme 75

Analysis of compound (**113**) indicated the elemental composition $C_{10}H_{11}NO_2$ which was in agreement with the mass spectrum (MH^+ 178, 55%).

The ^1H NMR spectrum for compound (**113**) showed a singlet at 1.61 ppm assigned to be the equivalent methyl groups, and a multiplet at 7.75 ppm assigned to

the aromatic protons.

It is probable that compound (**113**) is a rapidly equilibrating mixture of the two invertomers shown in (**Figure 35**).

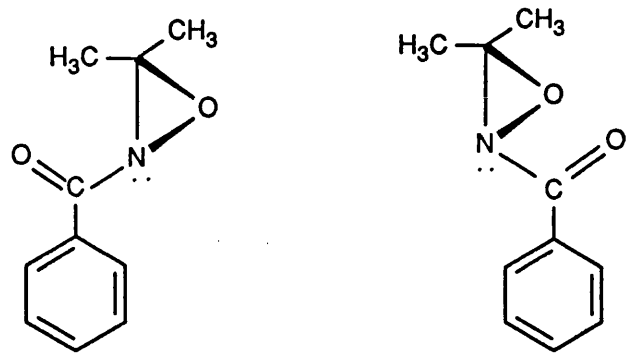
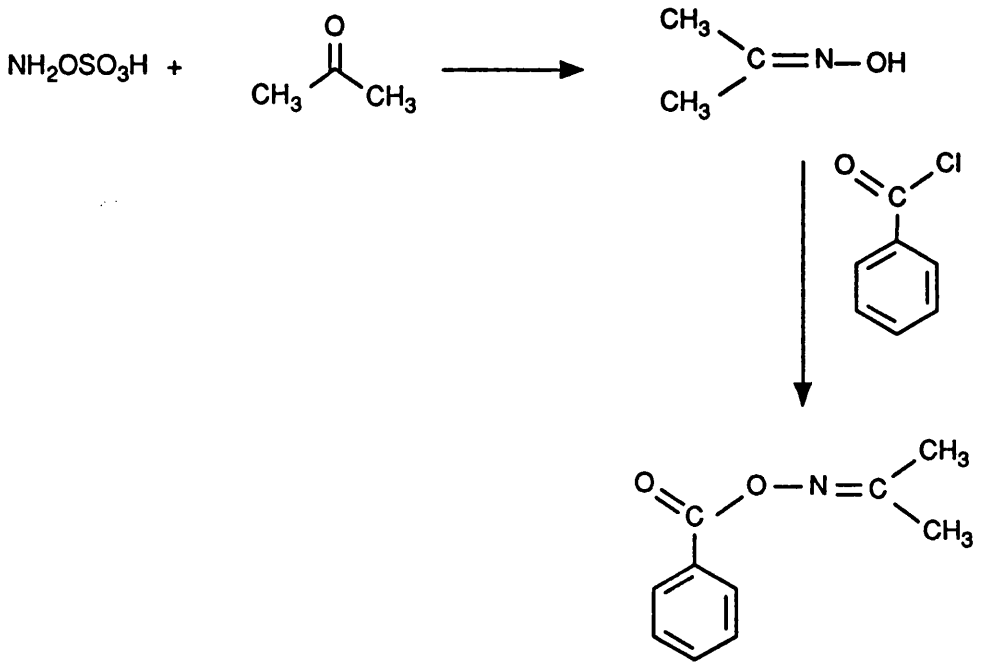


Figure 35

The oxaridine ring carbon exhibited the chemical shift in the range of 75-100 ppm [91]. This range for the chemical shift may be attributed to the difference in substitution on the oxaziridine rings.

The ^{13}C NMR of compound (**113**) showed a singlet at 84.51 ppm which confirmed the presence of a oxaziridine function in the compound. The formation of oxime instead of oxaziridine had to be considered (**Scheme 76**), since the ^{1}H NMR, mass spectrum and elemental analysis for compound (**113**) (oxaziridine) and compound (**117**) (oxime) could be the same. However, the resonance of the C=N group in the ^{13}C NMR normally appears at 155-165 ppm. The IR does not help in elucidating the correct structure, since the signal of C=N for oximes is very weak. However, the appearance of the methyl groups as a singlet precluded structure (**117**), supported by the absence of a C=N absorption in the ^{13}C NMR spectrum.

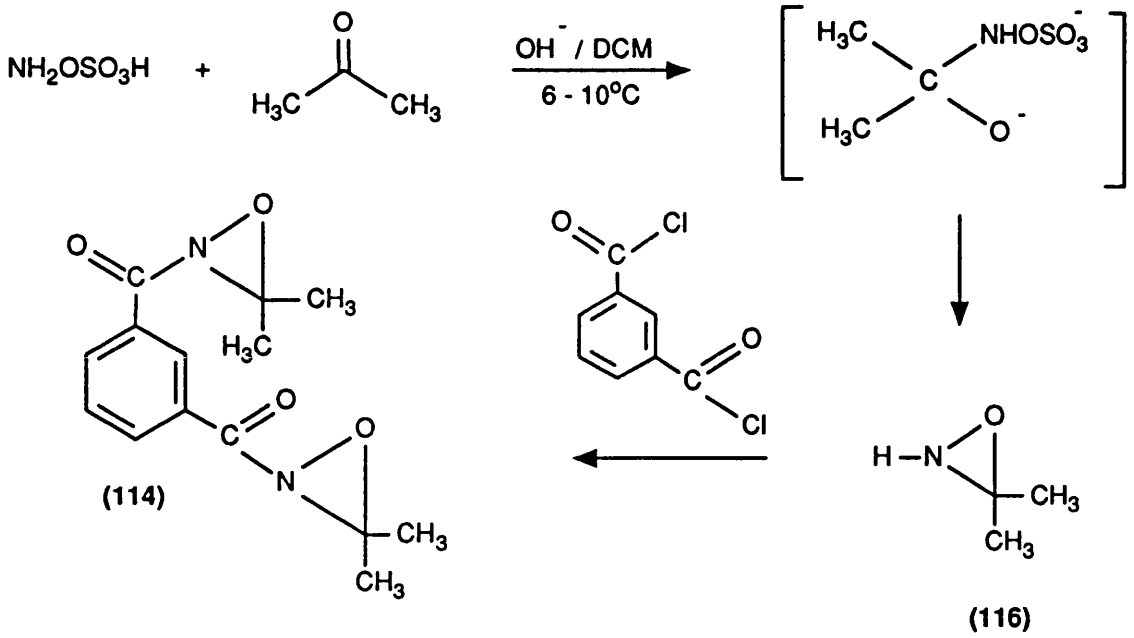


(117)

Scheme 76

5.3 Synthesis of 1,3-Benzenedicarbonyl-bis (3,3-dimethyloxaziridine) (114)

1,3-Benzenedicarbonyl-bis (3,3-dimethyloxaziridine) (**114**) was also prepared according to the general procedure of Schmitz *et al.*[98] for the preparation of oxaziridines. Hydroxylamine-O-sulfonic acid reacted with acetone in 2N sodium hydroxide solution to form 3,3-dimethyl oxaziridine (**116**); which was reacted *in situ* with isophthaloyl dichloride yielding 1,3-Benzenedicarbonyl-bis (3,3-dimethyloxaziridine) (**114**) (**Scheme 77**).



Scheme 77

The bis-oxaziridine (**114**) was isolated as a crystalline solid in 10% yield. Recrystallization of the compound (**114**) from ethyl acetate and light petroleum ether (b.p. 60-80°C), gave crystals of suitable quality for a successful X-ray crystallographic structure determination (**Figure 36**). Full details are included in **Appendix 1.**

Analysis of compound (**114**) indicated the elemental composition $C_{14}H_{16}N_2O_4$, which was in agreement with the mass spectrum (MH^+ 277, 25%).

The ^1H NMR spectrum exhibited one singlet at 1.65 ppm for the methyl group from both oxaziridine rings. This indicates that the oxaziridine ring is conformationally mobile and can undergo a "flipping" movement that interconverts the nitrogen lone pair from one side of the XYZ plane to the other, thus converting the molecule between its enantiomers faster than the NMR time scale (**Figure 37**).

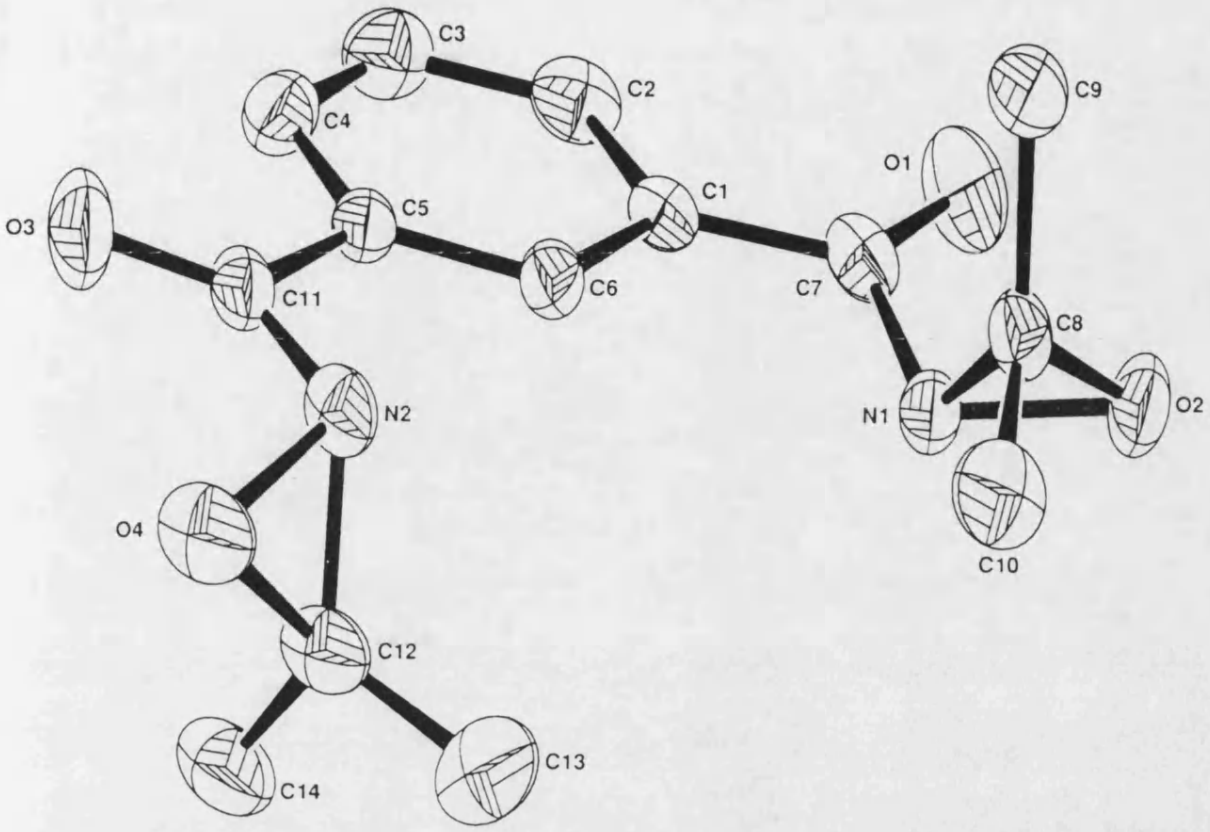


Figure 36 - ORTEP diagram of compound **(114)**

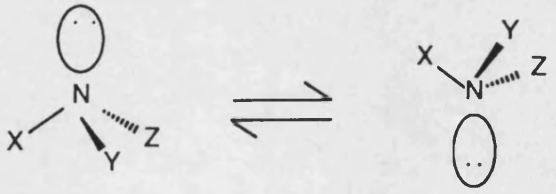
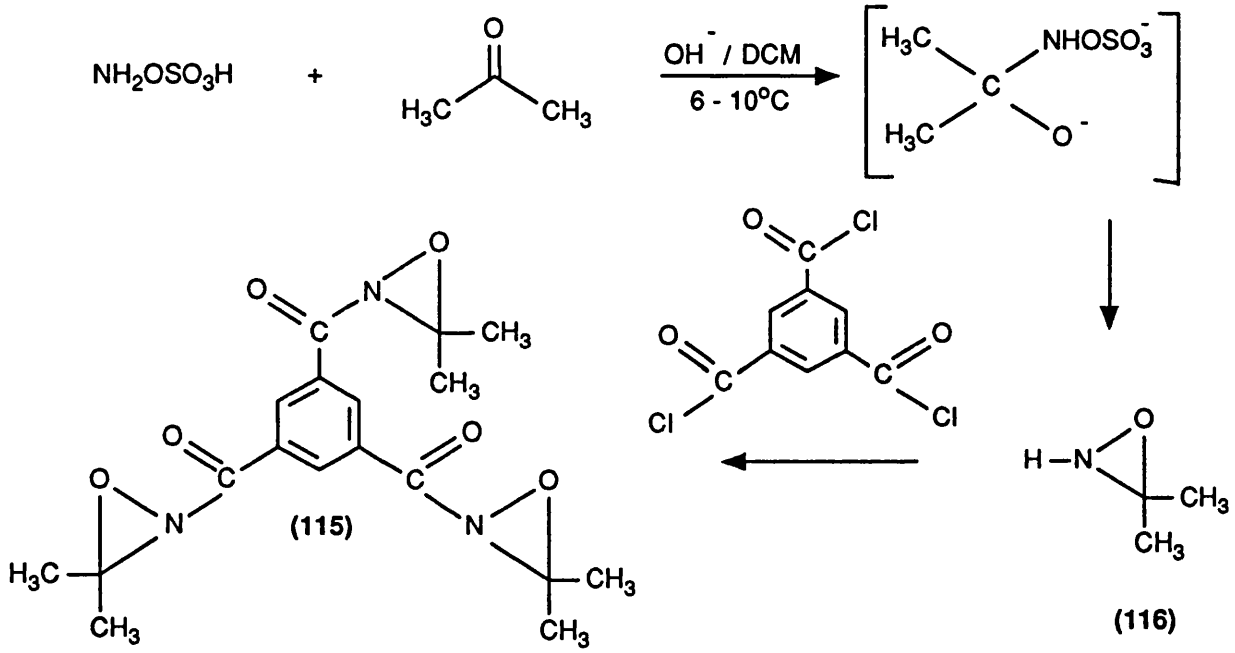


Figure 37

5.4 Synthesis of 1,3,5-Benzenetricarbonyl-tris (3,3-dimethyl oxaziridine) (115)

1,3,5-Benzenetricarbonyl-tris (3,3-dimethyloxaziridine) (**115**) was prepared according to the procedure of Schmitz *et al.*[98] for the preparation of oxaziridines.

Hydroxylamine-O-sulfonic acid reacted with acetone in sodium hydroxide 2N solution, to form 3,3-dimethyl oxaziridine (**116**), which was reacted *in situ* with 1,3,5-benzene tricarbonyl trichloride yielding 1,3,5-Benzenetricarbonyl-tris (3,3-dimethyloxaziridine) (**115**) (**Scheme 78**).



Scheme 78

The compound (**115**) was isolated as a white solid in 10% yield. Analysis of compound (**115**) indicated the elemental composition $C_{18}H_{21}N_3O_6$, which was in agreement with the mass spectrum (MH^+ 376, 100%). The ^1H NMR spectrum exhibited

one singlet at 1.68 ppm for the methyl groups from both oxaziridine rings. This indicates a "flipping" movement of the oxaziridine rings faster than the NMR time scale, as was explained for the compound (**114**).

5.5 The Reactivity of Oxaziridines in Oxidation Reactions

F. A. Davis[93] reported the oxidation of sulfides and alkenes by 2-sulfonyl oxaziridines. Boyd and Jennings[109] described the synthesis of *N*-phosphinoyl oxaziridines, and also reported that these oxaziridines epoxidize alkenes, and oxidize sulfides to sulfoxides.

The oxaziridines (**113**), (**114**) and (**115**) may also be capable of oxidising sulfides to sulfoxides and epoxidising alkenes. Thus, the compounds (**113**), (**114**), and (**115**) were tested in the oxidative reactions of sulfides to sulfoxides, styrene to styrene oxide, cyclohexene to cyclohexene oxide, and 1-(2-cyclohexenyl)-2-propanone to [1-(2-cyclohexenyl)-2-propanone] oxide (**Scheme 79**).

It was verified that the compounds (**113**), (**114**) and (**115**) exhibited similar reactivities in these oxidative reactions.

The oxidation of methyl tolyl sulfide proceeded successfully in 60% yield. The reaction was monitored by TLC analysis, and was complete in 6h at r.t., using $CHCl_3$ as solvent. The presence of a polar spot, when the reaction mixture was run in petrol/ethyl acetate (7:3), identical to the standard sulfoxide (from Aldrich), revealed the success of the oxidative reaction. The ^1H NMR analysis and mass spectrum of the resulting compound confirmed the formation of methyl tolyl sulfoxide. A strong band at 1039 cm^{-1} in the IR spectra also identified the presence of a sulfoxide.

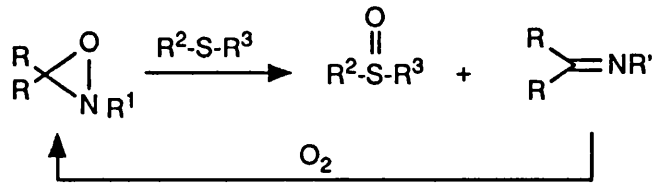
The epoxidation of styrene was not a very successful reaction. Only a low yield of styrene oxide was formed when the reaction was refluxed overnight in chloroform.

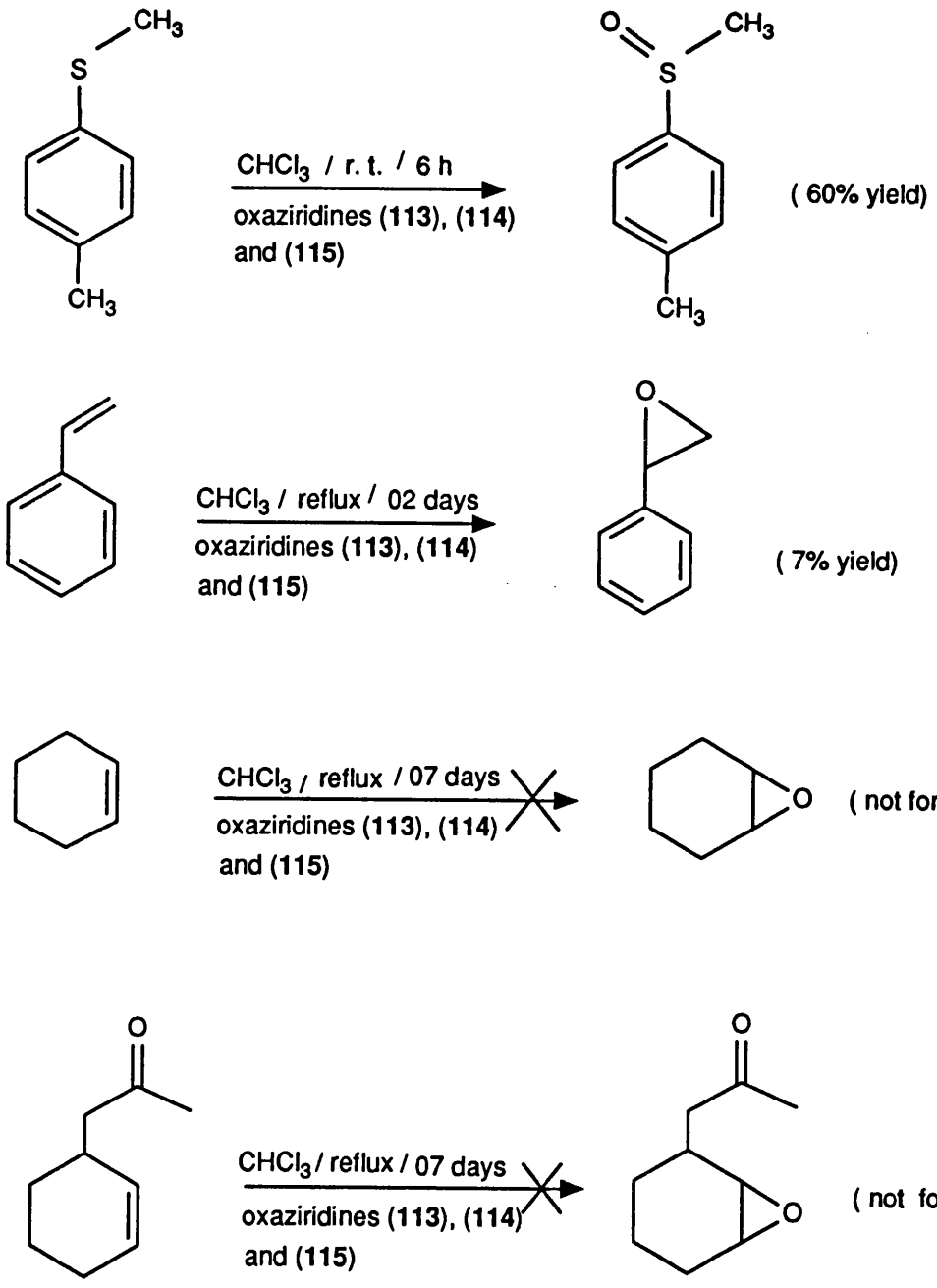
The reaction failed completely for the epoxidation of cyclohexene and 1-(2-cyclohexenyl)-2-propanone, only starting materials were observed after 7 days

refluxing in chloroform.

The mono- bis- and tris-oxaziridines did not differ much in their oxidizing abilities, as monitored by TLC. It had been hoped that the last of these would display superior oxidizing abilities, but this was not realized.

Future studies should possibly address the possibility of developing a catalytic system for oxidation, employing catalytic quantities of oxaziridine.




CH3
S
CH3
CHCl3 / r. t. / 6 h
oxaziridines (113), (114)
and (115)
O
CH3
S
CH3
(60% yield)
CHCl3 / reflux / 02 days
oxaziridines (113), (114)
and (115)
O
(7% yield)
CHCl3 / reflux / 07 days
oxaziridines (113), (114)
and (115)
O
(not formed)
O
CHCl3 / reflux / 07 days
oxaziridines (113), (114)
and (115)
O
O
(not formed)

Scheme 79

CHAPTER SIX

EXPERIMENTAL

CHAPTER SIX - EXPERIMENTAL

6.1 Experimental Procedure

2-Benzoyl-3,3-dimethyloxaziridine (113)

A solution of hydroxylamine-O- sulfonic acid (0.01mol, 1.12g) in water (10ml) and NaOH 2N (5ml), was added rapidly to a stirred solution of acetone (13.6mmol, 1.0ml) in dichloromethane (30ml) and NaOH 2N (10ml) at 2-5°C. Immediately , a solution of benzoyl chloride (0.01mol, 1.16ml) in dichloromethane (10ml) was added, and the stirring was continued for an additional 10 min. The dichloromethane layer was then separated and dried over magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator gave an oil which was chromatographed on a silica gel column using petrol/ethyl acetate (v/v 7:3) as the eluent. Removal of the solvents on a rotating evaporator afforded 0.26g (15% yield) of compound (**113**) as a pale yellow oil; R_f= 0.50(petrol-ethyl acetate 4:1); ν_{max}(liquid film)/cm^{-1} 3063, 3000, 2943, 1715(C=O), 1598, 1581, 1450(oxaz.), 1384, 1324, 1240(P=O), 1178, 1120, 1089, 1070, 1000, 846, 737, 659, 634; $\delta_H(CDCl_3)$ 1.61(s, $C\underline{H}_3$), 7.47-8.01(m, Ar-$\underline{H}$); $\delta_C(CDCl_3)$ 21.94(s, C-$\underline{C}H_3$), 84.51(s, $C_{oxaz.}$), 128.54, 128.73, 131.28, 133.81(4s, Ar), 177.35(s, C=O); m/z(C.I.) 178(MH^+, 55%), 162(M^+ - CH_3, 15), 105(M^+ - $NOC(CH_3)_2$, 100), 85(20). Anal. Calcd. for $C_{10}H_{11}NO_2$: C, 67.8; H, 6.2; N, 7.9; Found: C, 67.4; H, 6.2; N, 7.9.

1,3-Benzenedicarbonyl-bis (3,3-dimethyloxaziridine) (114)

A solution of hydroxylamine-O- sulfonic acid (0.01mol, 1.12g) in water (10ml) and NaOH 2N (5ml), was added rapidly to a stirred solution of acetone (13.6mmol, 1.0ml) in dichloromethane (30ml) and NaOH 2N (20ml) at 2-5°C. Immediately , a

solution of isophthaloyl dichloride (0.005mol, 1.0g) in dichloromethane (10ml) was added, and the stirring was continued for an additional 10 min. The dichloromethane layer was then separated and dried over magnesium sulphate and filtered. Concentration on a rotating evaporator gave a white solid which was recrystallised from ethyl acetate/light petroleum (b.p. 60-80°C) to give colourless crystals (0.16g, 6.0% yield): m.p. 114-116°C; R_f= 0.27(petrol-ethyl acetate 4:1); $\nu_{max}(CDCl_3)/cm^{-1}$ 2979, 2935, 1716(C=O), 1602, 1449, 1432(oxaz.), 1384, 1325, 1251, 1200(P=O), 1115, 1024, 1002, 731, 647; $\delta_C(CDCl_3)$ 1.65(s, $C\underline{H}_3$), 7.66-8.61(m, Ar-$\underline{H}$); $\delta_C(CDCl_3)$ 22.14(s, C-$\underline{C}H_3$), 85.12(s, $C_{oxaz.}$), 128.97, 129.54, 132.07, 133.58(4s, Ar), 176.56(s, C=O); m/z(C.I.) 277(MH^+,25%); m/z(E.I.) 276(M^+, 2%), 204(M^+ - $NOC(CH_3)_2$, 90), 146(62), 118(100), 90(78), 43(66). Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.8, H, 5.8; N, 10.1; Found: C, 60.6, H, 5.8; N, 10.0.

1,3,5-Benzenetricarbonyl-tris (3,3-dimethyloxaziridine) (115)

A solution of hydroxylamine-O-sulfonic acid (0.01mol, 1.12g) in water (10ml) and NaOH 2N(5ml), was added rapidly to a stirred solution of acetone (13.6mmol, 1.0ml) in dichloromethane (30ml) and NaOH 2N(20ml) at 2-5°C. Immediately, a solution of 1,3,5-benzene tricarbonyl trichloride (3.3mmol, 0.85g) in dichloromethane (10ml) was added, and the stirring was continued for an additional 10min. The dichloromethane layer was then separated and dried over magnesium sulphate and filtered. Concentration on a rotating evaporator gave a white solid which was chromatographed on a silica gel column using petrol/ethyl acetate (v/v 7:3) as the eluent. Removal of the solvents on a rotating evaporator afforded 0.13g (10% yield) of compound (**115**) as a white solid; m.p. 110°C; R_f= 0.62(petrol-ethyl acetate 1:1); $\nu_{max}(CDCl_3)/cm^{-1}$ 2995, 1720(C=O), 1623, 1450(oxaz.), 1385, 1326, 1251, 1217, 1162, 1115, 990, 908, 813, 732, 649; $\delta_H CDCl_3$) 1.68(s, $C\underline{H}_3$), 8.83(s, Ar-$\underline{H}$); $\delta_C(CDCl_3)$ δ 22.34ppm (s, C-$\underline{C}H_3$), 85.74(s, $C_{oxaz.}$), 132.92, 133.42(2s, Ar), 175.88(s, C=O);

m/z(C.I.) 376(MH^+,100%), 360(M^+-CH_3, 35) 303(M^+-$NOC(CH_3)_2$,65); m/z(E.I.) 375(M^+, 4%). Anal. Calcd. for $C_{18}H_{21}N_3O_6$: C, 57.6, H, 5.64; N, 11.19; Found: C, 57.4, H, 5.65; N, 11.2.

Oxidation of Methyl p-Tolyl Sulfide

To the solution of methyl p-tolyl sulfide (29.5mg, 0.21mmol) in chloroform (5ml) was added 1,3,5-benzenetricarbonyl)-tris (3,3-dimethyloxaziridine) (**115**) (80.1mg, 0.21mmol), and it was left stirring overnight. The solvent was evaporated under reduced pressure, and the product was purified using column chromatography on silica gel with petrol-ethyl acetate (1:1) as the eluent, to give the methyl p-tolylsulfoxide as a pale oil in 60.0% yield; Rf= 0.29 (petrol-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2999, 1597, 1494, 1411, 1215, 1088, 1039(S=O), 1015, 955, 754, 666; δ_H($CDCl_3$) 2.39(s, Ar-H), 2.68(s, S-CH_3), 7.29-7.55(Ar-H); m/z (E.I.) 154(M^+, 70%), 139(100), 111(15), 91(30).

The reaction was repeated using 1,3-benzenedicarbonyl-bis (3,3-dimethyl oxaziridine) (**114**) and 2-benzoyl-3,3-dimethyloxaziridine (**113**), and similar results were obtained.

Oxidation of Styrene

To the solution of styrene (0.10g, 0.96mmol) in chloroform (6ml) was added 1,3-benzenedicarbonyl-bis (3,3-dimethyloxaziridine) (**114**)(0.276g, 0.99mmol). It was refluxed for 2 weeks, then the solvent was removed under reduced pressure and the product was purified using column chromatography on silica gel with petrol-ethyl acetate (9:1) as the eluente to give the styrene oxide in 7.6% yield; Rf= 0.75 (petrol-ethyl acetate 9:1); ν_{max}(liquid film)/cm^{-1} 2924, 1703, 1454, 1259(epoxide), 1202, 1024(epoxide), 876, 801(epoxide), 757, 698; δ_H($CDCl_3$) 2.73(dd, CH_2, $J_{Ha,Ha'}$=

30Hz, $J_{Ha,Hb}$= 14Hz), 3.06(dd, C$\underline{H}_2$, $J_{Ha',Ha}$= 30Hz, $J_{Ha',Hb}$= 22Hz), 3.79(dd, C$\underline{H}$, $J_{Hb,Ha}$= 14Hz, $J_{Hb,Ha'}$= 22Hz), 7.22-7.34(m, Ar-$\underline{H}$).

The reaction was repeated with 2-benzoyl-3,3-dimethyloxaziridine (**113**) and 1,3,5-benzenetricarbonyl-tris (3,3-dimethyloxaziridine) (**115**) and similar results were obtained.

Attempted Preparation of tris [3,3-dimethyl oxaziridine-2-yl] phosphine oxide (117)

A solution of hydroxylamine-O-sulfonic acid (0.01mol, 1.12g) in water (10ml) and NaOH 2N(5ml), was added rapidly to a stirred solution of acetone (13.6mmol, 1.0ml) in dichloromethane (30ml) and NaOH 2N(20ml) at 0°C. Immediately, a solution of phosphorus oxychloride (0.51g, 3.3mmol) in dichloromethane (10ml) was added, and the stirring was continued for an additional 10min. T.L.C. analysis (silica gel, petrol-ethyl acetate 1:1) using PMA indicated a complex mixture of products with only a trace of an oxidant. Some others solvents such as benzene, diethyl ether, THF and toluene were also tested without success.

Attempted Preparation of 2-Diethoxyphosphoryl-3,3-dimethyl oxaziridine (118)

Method A

A solution of hydroxylamine-O-sulfonic acid (0.01mol, 1.12g) in water (10ml) and NaOH 2N(5ml), was added rapidly to a stirred solution of acetone (13.6mmol, 1.0ml) in dichloromethane (30ml) and NaOH 2N(20ml) at 0°C. Immediately, a solution of diethyl phosphorochloridate (0.51g, 3.3mmol) in dichloromethane (10ml) was added, and the stirring was continued for an additional 10min. Then the reaction was analyzed by TLC (silica gel, petrol-ethyl acetate 1:1) using PMA. No oxidant was

detected.

Method B

Acetone (0.01mol, 0.8ml) was added to a stirred solution of diethylphosphoramidate (0.01mol, 1.53g) in ether (20ml) at 0°C. The solution was allowed to warm to room temperature. Then a solution of mCPBA (50-60%)(0.01mol, 4.0g) in ether (10ml) was added at 0°C. After stirring for 2h at room temperature, the reaction mixture, was analyzed by TLC (silica gel, petrol-ethyl acetate 1:1) using PMA, but no oxidant was detected.

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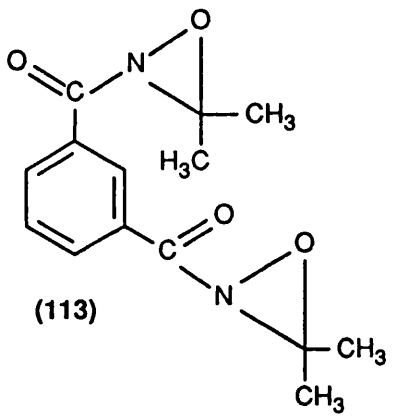
APPENDICES

APPENDIX ONE

X-ray Crstallographic Data for (113)



(113)

Compound (**113**) was crystallised from ethyl acetate/light petroleum. A crystal of approximate dimensions 0.2x0.2x0.5 mm was used for data collection.

Crystal Data: $C_{14}H_{16}N_2O_4$, m = 276.4, orthorhombic, a = 5.701 (1), b = 7.968 (1), c = 31.447Å(4), U = 1428.5 $Å^3$, space group $P2_12_12_1$, Z = 4, D_C= 1.33 gcm^{-3}, μ(Mo-Kα) = 0.90 cm^{-1}, F(000) = 624. Data were measured at room temperature on a CAD4 automatic four-circle diffractometer in the range $2 \leq \theta \leq 24°$. 1640 reflections were collected of which 1014 were unique with $I \geq 2\sigma(I)$. Data were corrected for Lorentz and polarization but not for absorption. The structure was solved by Direct methods and refined using the SHELX[1,2] suite of programs. In the final least squares cycles all atoms were allowed to vibrate anisotropically. Hydrogen atoms were included at calculated positions. Final residuals after 10 cycles of least squares were R = 0.0449, R_w= 0.0471, for a weighting scheme of $w = 1.7482/[\sigma^2(F)+0.000584(F)^2]$. Max. final shift/esd was 0.007. The max. and min. residual densities were 0.09 and -0.08 $eÅ^{-3}$ respectively. Final fractional atomic coordinates and isotropic thermal parameters,

anisotropic temperature factor, hydrogen fractional atomic co-ordinates and isotropic temperature factors, bond lengths, bond angles and selected non-bonded distances are given in Tables 1 to 7, respectively. The asymmetric unit is shown in Figure 1, along with the labelling scheme used.

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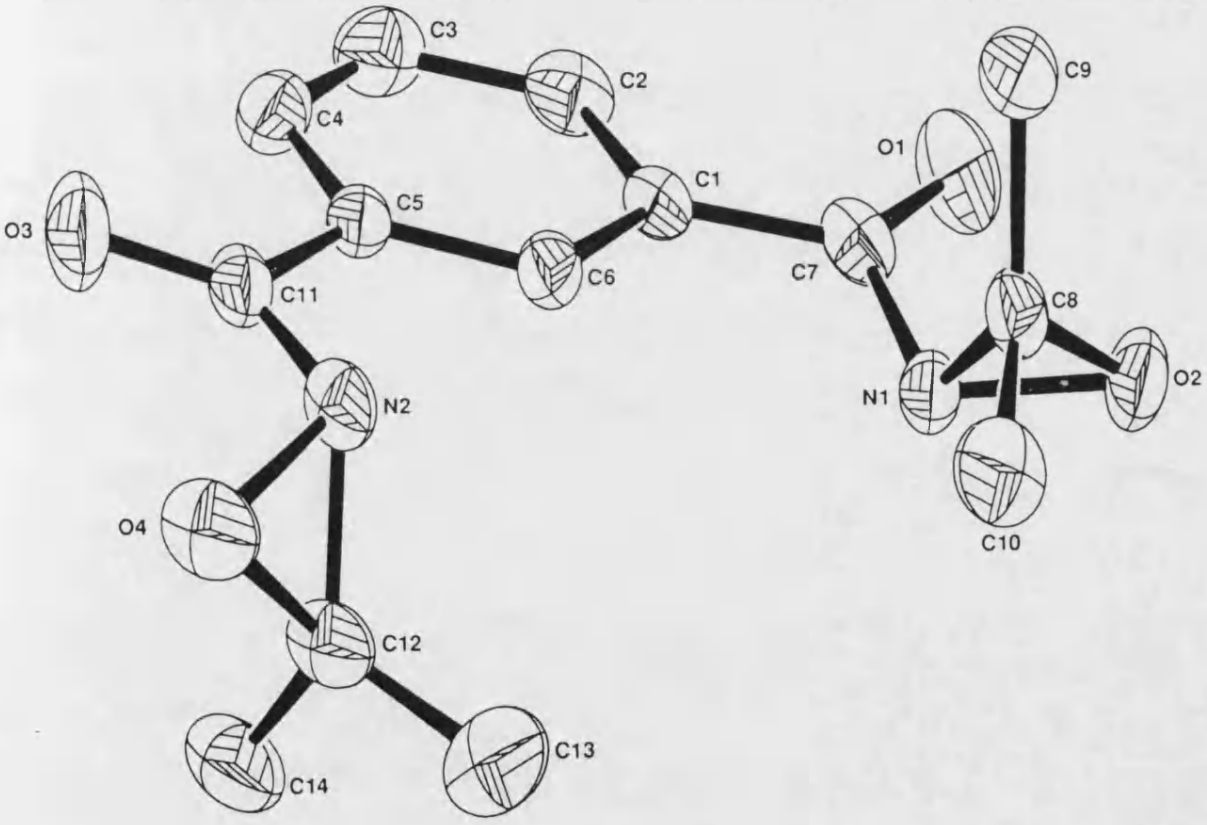


Figure 36

Table 1 Fractional atomic co-ordinates ($x10^3$) and equivalent isotropic temperature factors ($Å^2x10^3$)

	x	y	z	U
O(1)	-427(7)	1884(5)	2267(1)	83(2)
O(2)	-1837(6)	3897(4)	1622(1)	71(1)
O(3)	9084(7)	-1333(5)	1038(1)	86(2)
O(4)	7287(6)	-77(6)	311(1)	82(2)
N(1)	-283(6)	2417(5)	1551(1)	49(1)
N(2)	6363(7)	446(5)	735(1)	56(1)
C(1)	2616(7)	605(5)	1875(1)	40(1)
C(2)	3296(9)	-413(6)	2216(2)	58(2)
C(3)	5192(11)	-1449(7)	2175(2)	71(2)
C(4)	6485(9)	-1481(6)	1806(2)	62(2)
C(5)	5838(8)	-480(6)	1464(2)	44(1)
C(6)	3878(8)	540(6)	1496(2)	45(2)
C(7)	597(9)	1708(6)	1933(2)	52(2)
C(8)	383(9)	4099(6)	1426(2)	56(2)
C(9)	2154(9)	5048(6)	1674(2)	69(2)
C(10)	318(12)	4360(7)	954(2)	83(2)
C(11)	7251(8)	-565(6)	1069(2)	56(2)
C(12)	4888(10)	-331(8)	419(2)	69(2)
C(13)	3175(10)	815(9)	207(2)	93(3)
C(14)	4119(11)	-2098(8)	466(2)	91(3)

Table 2 Fractional atomic co-ordinates ($x10^3$)

	x	y	z
O(1)	-427(7)	1884(5)	2267(1)
O(2)	-1837(6)	3897(4)	1622(1)
O(3)	9084(7)	-1333(5)	1038(1)
O(4)	7287(6)	-77(6)	311(1)
N(1)	-283(6)	2417(5)	1551(1)
N(2)	6363(7)	446(5)	735(1)
C(1)	2616(7)	605(5)	1875(1)
C(2)	3296(9)	-413(6)	2216(2)
C(3)	5192(11)	-1449(7)	2175(2)
C(4)	6485(9)	-1481(6)	1806(2)
C(5)	5838(8)	-480(6)	1464(2)
C(6)	3878(8)	540(6)	1496(2)
C(7)	597(9)	1708(6)	1933(2)
C(8)	383(9)	4099(6)	1426(2)
C(9)	2154(9)	5048(6)	1674(2)
C(10)	318(12)	4360(7)	954(2)
C(11)	7251(8)	-565(6)	1069(2)
C(12)	4888(10)	-331(8)	419(2)
C(13)	3175(10)	815(9)	207(2)
C(14)	4119(11)	-2098(8)	466(2)

Table 3 Anisotropic temperature factors (Åx10^3)

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O(1)	95(3)	83(3)	70(3)	-7(2)	38(2)	5(3)
O(2)	42(2)	60(2)	113(3)	-11(2)	2(2)	11(2)
O(3)	59(2)	73(3)	127(4)	-22(2)	15(2)	17(3)
O(4)	60(2)	120(3)	67(2)	-28(2)	31(2)	-21(3)
N(1)	40(2)	42(2)	65(3)	-7(2)	1(2)	4(2)
N(2)	51(2)	63(2)	54(3)	-10(2)	18(2)	-4(2)
C(1)	46(2)	38(2)	37(2)	-2(2)	5(2)	-8(3)
C(2)	76(4)	45(3)	53(3)	4(3)	-4(3)	-16(3)
C(3)	88(4)	56(3)	70(4)	10(3)	-25(4)	-4(4)
C(4)	55(3)	45(3)	85(4)	-4(3)	-19(3)	1(3)
C(5)	38(2)	37(2)	57(3)	-7(3)	-2(2)	-5(3)
C(6)	43(2)	40(2)	52(3)	-5(2)	0(2)	1(2)
C(7)	60(3)	46(3)	49(3)	-9(3)	12(3)	-6(3)
C(8)	58(3)	38(3)	72(4)	-7(2)	0(3)	8(3)
C(9)	64(3)	48(3)	94(4)	-10(3)	-9(3)	-6(3)
C(10)	114(5)	54(3)	82(4)	13(3)	-14(4)	6(4)
C(11)	37(3)	45(3)	86(4)	-22(3)	3(3)	-3(3)
C(12)	52(3)	98(4)	58(3)	-29(3)	20(3)	-18(4)
C(13)	68(4)	145(6)	65(4)	2(4)	8(3)	-2(5)
C(14)	72(4)	99(5)	103(5)	-48(4)	17(4)	-25(4)

The temperature factor exponent takes the form:

$-2\ (U\ .h\ .a^*\ +\ \ldots\ \ +2U\ .h.k.a^*.b^*)$

Table 4 Hydrogen fractional atomic co-ordinates ($x10^3$) and isotropic temperature factors ($Å^2x10^3$)

	x	y	z	U
H(21)	2436(9)	-384(6)	2478(2)	133(6)
H(31)	5634(11)	-2162(7)	2403(2)	133(6)
H(41)	7828(9)	-2202(6)	1783(2)	133(6)
H(61)	3397(8)	1206(6)	1257(2)	133(6)
H(91)	3583(9)	5118(6)	1513(2)	133(6)
H(92)	2450(9)	4479(6)	1938(2)	133(6)
H(93)	1583(9)	6159(6)	1731(2)	133(6)
H(101)	1877(12)	4494(7)	844(2)	133(6)
H(102)	-584(12)	5349(7)	893(2)	133(6)
H(103)	-410(12)	3406(7)	823(2)	133(6)
H(131)	1614(10)	544(9)	298(2)	133(6)
H(132)	3285(10)	684(9)	-96(2)	133(6)
H(133)	3528(10)	1955(9)	282(2)	133(6)
H(141)	2483(11)	-2130(8)	538(2)	133(6)
H(142)	5013(11)	-2626(8)	688(2)	133(6)
H(143)	4367(11)	-2685(8)	204(2)	133(6)

Table 5 Bond lengths (Å)

C(7)-O(1)	1.211(6)	N(1)-O(2)	1.492(6)
C(8)-O(2)	1.418(7)	C(11)-O(3)	1.215(6)
N(2)-O(4)	1.493(6)	C(12)-O(4)	1.424(7)
C(7)-N(1)	1.418(7)	C(8)-N(1)	1.448(7)
C(11)-N(2)	1.417(7)	C(12)-N(2)	1.443(7)
C(2)-C(1)	1.398(7)	C(6)-C(1)	1.393(6)
C(7)-C(1)	1.460(7)	C(3)-C(2)	1.367(8)
C(4)-C(3)	1.377(8)	C(5)-C(4)	1.388(7)
C(6)-C(5)	1.385(7)	C(11)-C(5)	1.482(8)
C(9)-C(8)	1.483(7)	C(10)-C(8)	1.498(8)
C(13)-C(12)	1.494(9)	C(14)-C(12)	1.483(10)

Table 6 Bond angles (°)

C(8)-O(2)-N(1)	59.6(4)	C(12)-O(4)-N(2)	59.2(4)
C(7)-N(1)-O(2)	113.5(5)	C(8)-N(1)-O(2)	57.7(4)
C(8)-N(1)-C(7)	120.4(5)	C(11)-N(2)-O(4)	112.1(5)
C(12)-N(2)-O(4)	58.0(4)	C(12)-N(2)-C(11)	118.4(6)
C(6)-C(1)-C(2)	119.4(5)	C(7)-C(1)-C(2)	118.2(5)
C(7)-C(1)-C(6)	122.4(5)	C(3)-C(2)-C(1)	119.9(6)
C(4)-C(3)-C(2)	120.9(6)	C(5)-C(4)-C(3)	120.1(6)
C(6)-C(5)-C(4)	119.7(5)	C(11)-C(5)-C(4)	118.5(6)
C(11)-C(5)-C(6)	121.7(5)	C(5)-C(6)-C(1)	120.0(5)
N(1)-C(7)-O(1)	121.2(6)	C(1)-C(7)-O(1)	123.9(6)
C(1)-C(7)-N(1)	114.5(5)	N(1)-C(8)-O(2)	62.7(4)
C(9)-C(8)-O(2)	115.9(5)	C(9)-C(8)-N(1)	120.5(5)
C(10)-C(8)-O(2)	115.1(6)	C(10)-C(8)-N(1)	113.1(5)
C(10)-C(8)-C(9)	117.8(6)	N(2)-C(11)-O(3)	122.2(6)
C(5)-C(11)-O(3)	124.0(6)	C(5)-C(11)-N(2)	113.6(5)
N(2)-C(12)-O(4)	62.8(4)	C(13)-C(12)-O(4)	115.8(6)
C(13)-C(12)-N(2)	115.3(6)	C(14)-C(12)-O(4)	116.3(7)
C(14)-C(12)-N(2)	120.7(7)	C(14)-C(12)-C(13)	115.6(6)

Table 7 Selected non-bonded distances (Å)

Intramolecular:

O(2)-O(1)	2.707	N(1)-O(1)	2.292
C(1)-O(1)	2.360	C(2)-O(1)	2.807

Intermolecular:

C(2)-O(1a)	3.157

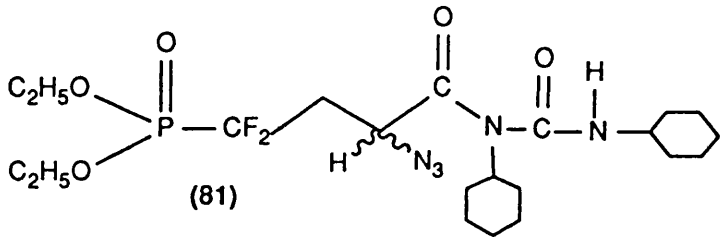
Key to symmetry operations relating designated atoms to reference atoms at (x,y,z):

(a) -x,-0.5+y,0.5-z

APPENDIX TWO

X-ray Crstallographic Data for (81)

Compound (**81**) was crystallised from ethyl acetate. A crystal of approximate dimensions 0.3x0.3x0.4 mm was used for data collection.



(81)

Crystal Data: $C_{21}H_{36}N_5O_5F_2P$, m = 507.5, triclinic, a = 11.294(3), b = 11.783(2), c= 12.011(3)Å, α = 105.62(2), β = 117.15(2), γ = 96.89(2)°, U = 1313.6Å^3, space group P1, Z = 2, D_c = 1.28 gcm^{-3}, μ(Mo-Kα) = 1.50 cm^{-1}, F(000) = 540. Data were measured at room temperature on a CAD4 automatic four-circle diffractometer in the range $2 \leq \theta \leq 24°$. 4359 reflections were collected of which 2472 were unique with $I \geq 2\sigma(I)$. Data were corrected for Lorentz and polarization but not for absorption. The structure was solved by Direct methods and refined using the SHELX[1,2] suite of programs. In the final least squares cycles all atoms were allowed to vibrate anisotropically. Hydrogen atoms were included at calculated positions, except in the instance H5 (attached to N5), which was located in an advanced Difference Fourier and refined at a distance of 0.96 Å from the parent atoms.

Examination of the supramolecular structure revealed that molecules dimerize about space group inversion centres as a result of a weak hydrogen bond between H5 of

one molecule and O3 of a lattice neighbour. (O3 - N5, 2.97 Å; O3 - H5, 2.02 Å).

Final residuals after 10 cycles of least squares were R = 0.0438, R_w= 0.0437, for a weighting scheme of w = $2.3075/[\sigma^2(F)+0.000686(F)^2]$. Max. final shift/esd was 0.003. The max. and min. residual densities were 0.10 and -0.10 $eÅ^{-3}$ respectively. Final fractional atomic coordinates and isotropic thermal parameters, bond distances and angles are given in Tables 8 to 14, respectively.

1. Sheldrick G. M., SHELX86, a computer program for crystal structure determination, University of Göttingen, 1986.

2. Sheldrick G. M., SHELX76, a computer program for crystal structure determination, University of Cambridge, 1976.

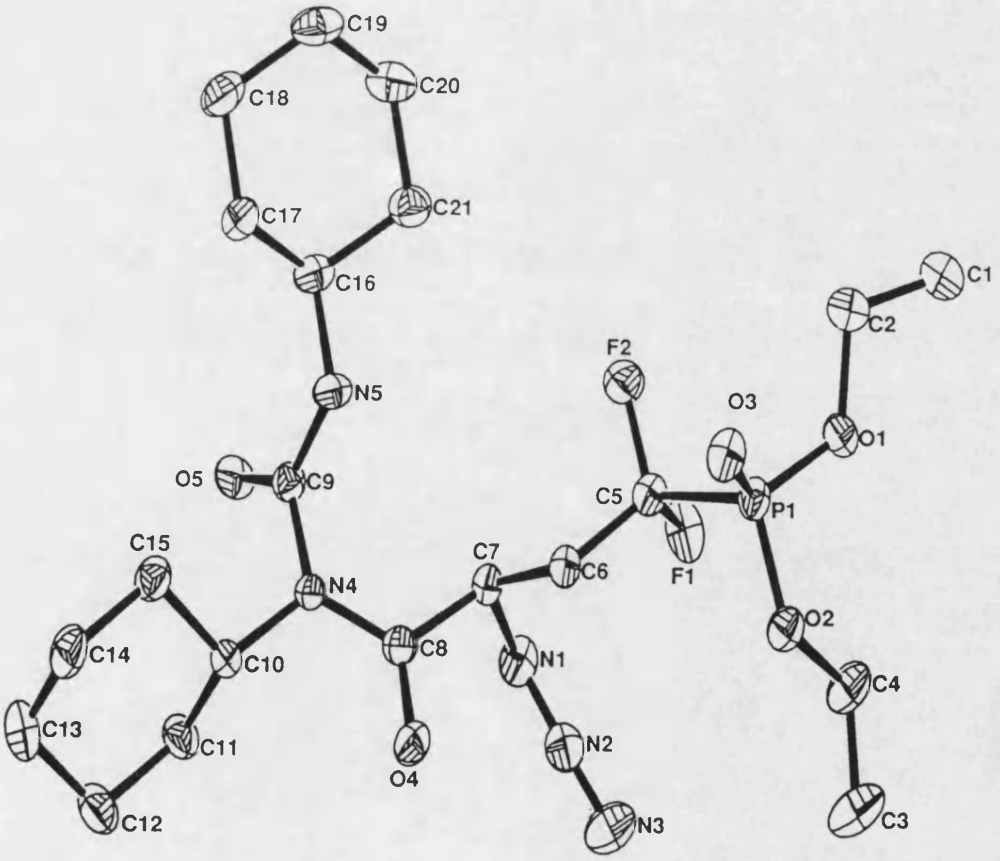


Figure 28

Table 8 Fractional atomic co-ordinates ($x10^4$) and equivalent isotropic temperature factors ($Å^2x10^3$)

	x	y	z	U
P(1)	3554(1)	7533(1)	4190(1)	42
F(1)	5381(2)	6897(2)	6021(2)	65(1)
F(2)	5256(2)	8749(2)	6787(2)	72(1)
N(1)	8187(3)	7371(3)	6772(3)	56(2)
N(2)	7797(3)	6607(3)	5662(4)	57(2)
N(3)	7451(4)	5798(3)	4699(4)	88(2)
N(4)	9899(2)	10379(2)	7556(2)	38(1)
N(5)	8904(3)	11339(2)	8728(2)	45(1)
O(1)	2500(2)	6844(2)	4446(2)	55(1)
O(2)	3629(2)	6529(2)	3109(2)	51(1)
O(3)	3287(2)	8604(2)	3840(2)	58(1)
O(4)	9051(3)	8816(2)	5586(2)	66(1)
O(5)	10995(2)	11000(2)	9874(2)	55(1)
C(1)	813(4)	6644(4)	5103(4)	79(2)
C(2)	1945(5)	7499(4)	5238(5)	84(3)
C(3)	3630(6)	4586(4)	1827(4)	89(3)
C(4)	3396(5)	5220(3)	2894(4)	69(2)
C(5)	5269(3)	7946(3)	5729(3)	44(2)
C(6)	6479(3)	8469(3)	5633(3)	40(1)
C(7)	7917(3)	8588(3)	6781(3)	43(2)
C(8)	9008(3)	9285(3)	6602(3)	44(1)
C(9)	9989(3)	10916(3)	8833(3)	41(2)
C(10)	11016(3)	10967(3)	7394(3)	41(2)
C(11)	12264(3)	10461(3)	7908(4)	55(2)

C(12)	13355(4)	11025(4)	7650(4)	69(2)
C(13)	13792(4)	12414(4)	8297(4)	77(3)
C(14)	12557(4)	12924(3)	7813(4)	65(2)
C(15)	11452(4)	12359(3)	8062(3)	53(2)
C(16)	8832(3)	12001(3)	9899(3)	49(2)
C(17)	9219(4)	13373(3)	10205(4)	63(2)
C(18)	9114(4)	14091(3)	11407(4)	71(2)
C(19)	7701(4)	13642(4)	11211(4)	67(2)
C(20)	7327(5)	12270(4)	10907(4)	70(3)
C(21)	7411(4)	11555(3)	9694(4)	57(2)

Table 9 Fractional atomic co-ordinates ($x10^4$)

	x	y	z
P(1)	3554(1)	7533(1)	4190(1)
F(1)	5381(2)	6897(2)	6021(2)
F(2)	5256(2)	8749(2)	6787(2)
N(1)	8187(3)	7371(3)	6772(3)
N(2)	7797(3)	6607(3)	5662(4)
N(3)	7451(4)	5798(3)	4699(4)
N(4)	9899(2)	10379(2)	7556(2)
N(5)	8904(3)	11339(2)	8728(2)
O(1)	2500(2)	6844(2)	4446(2)
O(2)	3629(2)	6529(2)	3109(2)
O(3)	3287(2)	8604(2)	3840(2)
O(4)	9051(3)	8816(2)	5586(2)
O(5)	10995(2)	11000(2)	9874(2)
C(1)	813(4)	6644(4)	5103(4)
C(2)	1945(5)	7499(4)	5238(5)
C(3)	3630(6)	4586(4)	1827(4)
C(4)	3396(5)	5220(3)	2894(4)
C(5)	5269(3)	7946(3)	5729(3)
C(6)	6479(3)	8469(3)	5633(3)
C(7)	7917(3)	8588(3)	6781(3)
C(8)	9008(3)	9285(3)	6602(3)
C(9)	9989(3)	10916(3)	8833(3)
C(10)	11016(3)	10967(3)	7394(3)
C(11)	12264(3)	10461(3)	7908(4)
C(12)	13355(4)	11025(4)	7650(4)

C(13)	13792(4)	12414(4)	8297(4)
C(14)	12557(4)	12924(3)	7813(4)
C(15)	11452(4)	12359(3)	8062(3)
C(16)	8832(3)	12001(3)	9899(3)
C(17)	9219(4)	13373(3)	10205(4)
C(18)	9114(4)	14091(3)	11407(4)
C(19)	7701(4)	13642(4)	11211(4)
C(20)	7327(5)	12270(4)	10907(4)
C(21)	7411(4)	11555(3)	9694(4)

Table 10 Anisotropic temperature factors ($Å \times 10^3$)

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
P(1)	34	38	45	15	13	11
F(1)	47(1)	71(1)	81(1)	54(1)	21(1)	17(1)
F(2)	54(1)	91(2)	49(1)	2(1)	24(1)	15(1)
N(1)	51(2)	54(2)	53(2)	23(2)	15(2)	20(1)
N(2)	53(2)	49(2)	74(2)	30(2)	31(2)	23(2)
N(3)	110(3)	55(2)	95(3)	17(2)	54(3)	31(2)
N(4)	32(1)	41(1)	34(1)	7(1)	15(1)	9(1)
N(5)	41(2)	54(2)	33(1)	10(1)	14(1)	24(1)
O(1)	44(1)	52(1)	69(2)	17(1)	31(1)	12(1)
O(2)	55(1)	43(1)	50(1)	15(1)	25(1)	10(1)
O(3)	53(1)	46(1)	60(1)	25(1)	13(1)	19(1)
O(4)	63(2)	62(2)	50(1)	-6(1)	33(1)	-9(1)
O(5)	40(1)	82(2)	38(1)	22(1)	14(1)	26(1)
C(1)	69(3)	86(3)	87(3)	23(2)	49(3)	22(2)
C(2)	72(3)	75(3)	97(3)	2(2)	56(3)	11(2)
C(3)	132(4)	56(2)	72(3)	14(2)	50(3)	32(3)
C(4)	89(3)	39(2)	68(2)	15(2)	37(2)	13(2)
C(5)	45(2)	39(2)	43(2)	17(2)	18(2)	15(2)
C(6)	37(2)	35(2)	43(2)	16(1)	15(1)	10(1)
C(7)	38(2)	40(2)	41(2)	9(1)	17(2)	6(1)
C(8)	37(2)	48(2)	38(2)	11(2)	16(2)	11(2)
C(9)	36(2)	42(2)	39(2)	13(1)	15(2)	11(1)
C(10)	38(2)	39(2)	44(2)	12(1)	22(2)	10(1)
C(11)	45(2)	51(2)	75(2)	25(2)	33(2)	19(2)
C(12)	48(2)	74(3)	91(3)	33(2)	39(2)	21(2)

C(13)	52(2)	77(3)	90(3)	27(2)	32(2)	-1(2)
C(14)	72(3)	40(2)	71(3)	15(2)	33(2)	1(2)
C(15)	55(2)	39(2)	56(2)	13(2)	26(2)	11(2)
C(16)	47(2)	57(2)	36(2)	14(2)	15(2)	28(2)
C(17)	57(2)	52(2)	61(2)	2(2)	27(2)	11(2)
C(18)	74(3)	58(2)	59(2)	3(2)	25(2)	25(2)
C(19)	80(3)	72(3)	59(2)	21(2)	39(2)	48(2)
C(20)	86(3)	77(3)	74(3)	31(2)	57(2)	37(2)
C(21)	66(2)	51(2)	63(2)	20(2)	39(2)	21(2)

Table 11 Hydrogen fractional atomic co-ordinates ($x1^4$) and isotropic temperature factors ($Å^2x10^3$)

	x	y	z	U
H(5)	8232(29)	11310(33)	7857(16)	85(2)
H(11)	452(4)	7074(4)	5622(4)	85(2)
H(12)	1142(4)	6017(4)	5425(4)	85(2)
H(13)	92(4)	6274(4)	4174(4)	85(2)
H(21)	1615(5)	8126(4)	4917(5)	85(2)
H(22)	2665(5)	7870(4)	6167(5)	85(2)
H(31)	3474(6)	3729(4)	1694(4)	85(2)
H(32)	4568(6)	4930(4)	2072(4)	85(2)
H(33)	3001(6)	4680(4)	1009(4)	85(2)
H(41)	2458(5)	4873(3)	2647(4)	85(2)
H(42)	4025(5)	5123(3)	3710(4)	85(2)
H(61)	6372(3)	7948(3)	4806(3)	85(2)
H(62)	6450(3)	9274(3)	5610(3)	85(2)
H(71)	7957(3)	9000(3)	7609(3)	85(2)
H(101)	10641(3)	10768(3)	6446(3)	85(2)
H(111)	11964(3)	9586(3)	7451(4)	85(2)
H(112)	12662(3)	10653(3)	8857(4)	85(2)
H(121)	12976(4)	10792(4)	6698(4)	85(2)
H(122)	14150(4)	10728(4)	8022(4)	85(2)
H(131)	14434(4)	12753(4)	8075(4)	85(2)
H(132)	14235(4)	12646(4)	9256(4)	85(2)
H(141)	12866(4)	13798(3)	8284(4)	85(2)
H(142)	12156(4)	12747(3)	6866(4)	85(2)

H(151)	10660(4)	12659(3)	7691(3)	85(2)
H(152)	11826(4)	12582(3)	9012(3)	85(2)
H(161)	9487(3)	11843(3)	10659(3)	85(2)
H(171)	10154(4)	13639(3)	10403(4)	85(2)
H(172)	8601(4)	13534(3)	9431(4)	85(2)
H(181)	9289(4)	14944(3)	11522(4)	85(2)
H(182)	9799(4)	13996(3)	12199(4)	85(2)
H(191)	7703(4)	14065(4)	12017(4)	85(2)
H(192)	7023(4)	13811(4)	10475(4)	85(2)
H(201)	7960(5)	12111(4)	11676(4)	85(2)
H(202)	6399(5)	12000(4)	10724(4)	85(2)
H(211)	6731(4)	11664(3)	8910(4)	85(2)
H(212)	7224(4)	10699(3)	9566(4)	85(2)

Table 12 Bond lengths (Å)

O(1)-P(1)	1.551(4)	O(2)-P(1)	1.546(4)
O(3)-P(1)	1.457(3)	C(5)-P(1)	1.851(5)
C(5)-F(1)	1.377(4)	C(5)-F(2)	1.373(4)
N(2)-N(1)	1.227(5)	C(7)-N(1)	1.500(6)
N(3)-N(2)	1.142(5)	C(8)-N(4)	1.353(5)
C(9)-N(4)	1.443(5)	C(10)-N(4)	1.483(5)
C(9)-N(5)	1.344(5)	C(16)-N(5)	1.456(5)
C(2)-O(1)	1.469(5)	C(4)-O(2)	1.464(5)
C(8)-O(4)	1.222(5)	C(9)-O(5)	1.214(4)
C(2)-C(1)	1.447(6)	C(4)-C(3)	1.453(6)
C(6)-C(5)	1.496(5)	C(7)-C(6)	1.535(6)
C(8)-C(7)	1.527(6)	C(11)-C(10)	1.526(6)
C(15)-C(10)	1.520(6)	C(12)-C(11)	1.522(7)
C(13)-C(12)	1.516(7)	C(14)-C(13)	1.515(7)
C(15)-C(14)	1.529(7)	C(17)-C(16)	1.518(6)
C(21)-C(16)	1.509(7)	C(18)-C(17)	1.529(7)
C(19)-C(18)	1.507(8)	C(20)-C(19)	1.517(7)
C(21)-C(20)	1.525(6)	H(5)-N(5)	0.960(2)
H(11)-C(1)	0.960	H(12)-C(1)	0.960
H(13)-C(1)	0.960	H(21)-C(2)	0.960
H(22)-C(2)	0.960	H(31)-C(3)	0.960
H(32)-C(3)	0.960	H(33)-C(3)	0.960
H(41)-C(4)	0.960	H(42)-C(4)	0.960
H(61)-C(6)	0.960	H(62)-C(6)	0.960
H(71)-C(7)	0.960	H(101)-C(10)	0.960
H(111)-C(11)	0.960	H(112)-C(11)	0.960
H(121)-C(12)	0.960	H(122)-C(12)	0.960

H(131)-C(13)	0.960	H(132)-C(13)	0.960
H(141)-C(14)	0.960	H(142)-C(14)	0.960
H(151)-C(15)	0.960	H(152)-C(15)	0.960
H(161)-C(16)	0.960	H(171)-C(17)	0.960
H(172)-C(17)	0.960	H(181)-C(18)	0.960
H(182)-C(18)	0.960	H(191)-C(19)	0.960
H(192)-C(19)	0.960	H(201)-C(20)	0.960
H(202)-C(20)	0.960	H(211)-C(21)	0.960
H(212)-C(21)	0.960		

Table 13 Bond angles (°)

O(2)-P(1)-O(1)	104.6(2)	O(3)-P(1)-O(1)	117.0(2)
O(3)-P(1)-O(2)	113.0(2)	C(5)-P(1)-O(1)	105.8(2)
C(5)-P(1)-O(2)	104.3(2)	C(5)-P(1)-O(3)	111.1(2)
C(7)-N(1)-N(2)	115.5(4)	N(3)-N(2)-N(1)	172.0(4)
C(9)-N(4)-C(8)	124.3(4)	C(10)-N(4)-C(8)	118.1(3)
C(10)-N(4)-C(9)	116.8(3)	C(16)-N(5)-C(9)	122.4(4)
C(2)-O(1)-P(1)	122.0(3)	C(4)-O(2)-P(1)	126.6(3)
C(1)-C(2)-O(1)	109.6(4)	C(3)-C(4)-O(2)	110.1(4)
F(1)-C(5)-P(1)	106.5(3)	F(2)-C(5)-P(1)	108.6(3)
F(2)-C(5)-F(1)	104.7(3)	C(6)-C(5)-P(1)	114.9(3)
C(6)-C(5)-F(1)	111.3(3)	C(6)-C(5)-F(2)	110.3(4)
C(7)-C(6)-C(5)	115.9(3)	C(6)-C(7)-N(1)	113.1(3)
C(8)-C(7)-N(1)	106.8(4)	C(8)-C(7)-C(6)	108.1(3)
O(4)-C(8)-N(4)	121.9(4)	C(7)-C(8)-N(4)	120.2(4)
C(7)-C(8)-O(4)	118.0(4)	N(5)-C(9)-N(4)	113.2(4)
O(5)-C(9)-N(4)	121.0(4)	O(5)-C(9)-N(5)	125.8(4)
C(11)-C(10)-N(4)	111.8(3)	C(15)-C(10)-N(4)	111.6(4)
C(15)-C(10)-C(11)	110.9(4)	C(12)-C(11)-C(10)	110.6(4)
C(13)-C(12)-C(11)	110.4(4)	C(14)-C(13)-C(12)	111.4(4)
C(15)-C(14)-C(13)	111.5(4)	C(14)-C(15)-C(10)	109.6(4)
C(17)-C(16)-N(5)	110.1(4)	C(21)-C(16)-N(5)	111.0(4)
C(21)-C(16)-C(17)	111.1(4)	C(18)-C(17)-C(16)	111.3(4)
C(19)-C(18)-C(17)	111.8(4)	C(20)-C(19)-C(18)	110.8(4)
C(21)-C(20)-C(19)	111.5(4)	C(20)-C(21)-C(16)	111.0(4)
C(9)-N(5)-H(5)	117.5(24)	C(16)-N(5)-H(5)	119.3(23)
H(12)-C(1)-H(11)	109.5	H(13)-C(1)-H(11)	109.5
H(13)-C(1)-H(12)	109.5	C(2)-C(1)-H(11)	109.5(3)

C(2)-C(1)-H(12)	109.5(4)	C(2)-C(1)-H(13)	109.4(4)
H(21)-C(2)-O(1)	109.5(3)	H(21)-C(2)-C(1)	109.5(4)
H(22)-C(2)-O(1)	109.4(3)	H(22)-C(2)-C(1)	109.4(4)
H(22)-C(2)-H(21)	109.5	H(32)-C(3)-H(31)	109.5
H(33)-C(3)-H(31)	109.5	H(33)-C(3)-H(32)	109.5
C(4)-C(3)-H(31)	109.5(3)	C(4)-C(3)-H(32)	109.5(4)
C(4)-C(3)-H(33)	109.5(3)	H(41)-C(4)-O(2)	109.3(3)
H(41)-C(4)-C(3)	109.3(4)	H(42)-C(4)-O(2)	109.3(3)
H(42)-C(4)-C(3)	109.3(3)	H(42)-C(4)-H(41)	109.5
H(61)-C(6)-C(5)	107.8(3)	H(62)-C(6)-C(5)	107.8(3)
H(62)-C(6)-H(61)	109.5	C(7)-C(6)-H(61)	107.8(3)
C(7)-C(6)-H(62)	107.8(3)	H(71)-C(7)-N(1)	108.4(3)
H(71)-C(7)-C(6)	107.2(3)	C(8)-C(7)-H(71)	113.4(3)
H(101)-C(10)-N(4)	106.8(2)	C(11)-C(10)-H(101)	107.6(3)
C(15)-C(10)-H(101)	107.8(3)	H(111)-C(11)-C(10)	109.2(3)
H(112)-C(11)-C(10)	109.2(3)	H(112)-C(11)-H(111)	109.5
C(12)-C(11)-H(111)	109.2(3)	C(12)-C(11)-H(112)	109.2(3)
H(121)-C(12)-C(11)	109.2(3)	H(122)-C(12)-C(11)	109.3(3)
H(122)-C(12)-H(121)	109.5	C(13)-C(12)-H(121)	109.2(3)
C(13)-C(12)-H(122)	109.3(3)	H(131)-C(13)-C(12)	109.0(3)
H(132)-C(13)-C(12)	109.0(3)	H(132)-C(13)-H(131)	109.5
C(14)-C(13)-H(131)	109.0(3)	C(14)-C(13)-H(132)	109.0(3)
H(141)-C(14)-C(13)	109.0(3)	H(142)-C(14)-C(13)	109.0(3)
H(142)-C(14)-H(141)	109.5	C(15)-C(14)-H(141)	109.0(3)
C(15)-C(14)-H(142)	109.0(3)	H(151)-C(15)-C(10)	109.4(3)

H(151)-C(15)-C(14)	109.4(3)	H(152)-C(15)-C(10)	109.4(3)
H(152)-C(15)-C(14)	109.4(3)	H(152)-C(15)-H(151)	109.5
H(161)-C(16)-N(5)	108.5(2)	C(17)-C(16)-H(161)	108.4(3)
C(21)-C(16)-H(161)	107.5(3)	H(171)-C(17)-C(16)	109.0(3)
H(172)-C(17)-C(16)	109.0(3)	H(172)-C(17)-H(171)	109.5
C(18)-C(17)-H(171)	109.0(3)	C(18)-C(17)-H(172)	109.0(3)
H(181)-C(18)-C(17)	108.9(3)	H(182)-C(18)-C(17)	108.9(3)
H(182)-C(18)-H(181)	109.5	C(19)-C(18)-H(181)	108.9(3)
C(19)-C(18)-H(182)	108.9(3)	H(191)-C(19)-C(18)	109.1(3)
H(192)-C(19)-C(18)	109.1(3)	H(192)-C(19)-H(191)	109.5
C(20)-C(19)-H(191)	109.1(3)	C(20)-C(19)-H(192)	109.1(3)
H(201)-C(20)-C(19)	109.0(3)	H(202)-C(20)-C(19)	109.0(3)
H(202)-C(20)-H(201)	109.5	C(21)-C(20)-H(201)	109.0(3)
C(21)-C(20)-H(202)	109.0(3)	H(211)-C(21)-C(16)	109.1(3)
H(211)-C(21)-C(20)	109.1(3)	H(212)-C(21)-C(16)	109.1(3)
H(212)-C(21)-C(20)	109.1(3)	H(212)-C(21)-H(211)	109.5

Table 14 Selected non-bonded distances (Å)

Intramolecular:

F(1)-P(1)	2.602	F(2)-P(1)	2.634
C(2)-P(1)	2.642	H(21)-P(1)	2.797
H(22)-P(1)	2.926	C(4)-P(1)	2.689
H(41)-P(1)	2.939	H(42)-P(1)	2.905
C(6)-P(1)	2.829	H(61)-P(1)	2.864
H(62)-P(1)	3.038	F(2)-F(1)	2.177
N(1)-F(1)	2.805	N(2)-F(1)	2.995
O(1)-F(1)	2.905	O(2)-F(1)	3.000
H(42)-F(1)	2.579	C(6)-F(1)	2.372
H(61)-F(1)	2.655	C(7)-F(1)	2.876
H(22)-F(2)	2.657	C(6)-F(2)	2.356
H(62)-F(2)	2.492	C(7)-F(2)	3.037
N(3)-N(1)	2.363	O(4)-N(1)	2.838
C(6)-N(1)	2.532	H(61)-N(1)	2.649
H(71)-N(1)	2.021	C(8)-N(1)	2.430
O(4)-N(2)	2.855	C(6)-N(2)	2.794
H(61)-N(2)	2.473	C(7)-N(2)	2.311
C(8)-N(2)	2.955	N(5)-N(4)	2.327
H(5)-N(4)	2.404	O(4)-N(4)	2.252
O(5)-N(4)	2.315	C(7)-N(4)	2.497
H(71)-N(4)	2.604	H(101)-N(4)	1.986
C(11)-N(4)	2.492	H(111)-N(4)	2.657
H(112)-N(4)	2.706	C(15)-N(4)	2.484
H(151)-N(4)	2.659	H(152)-N(4)	2.695
O(5)-N(5)	2.278	C(7)-N(5)	3.145

H(71)-N(5)	2.555	C(8)-N(5)	3.068
H(161)-N(5)	1.983	C(17)-N(5)	2.439
H(171)-N(5)	2.634	H(172)-N(5)	2.612
C(21)-N(5)	2.445	H(211)-N(5)	2.623
H(212)-N(5)	2.642	C(9)-H(5)	1.980
C(16)-H(5)	2.101	C(21)-H(5)	2.719
O(2)-O(1)	2.450	O(3)-O(1)	2.565
C(1)-O(1)	2.382	H(12)-O(1)	2.566
H(13)-O(1)	2.566	H(21)-O(1)	2.005
H(22)-O(1)	2.004	C(4)-O(1)	2.903
H(41)-O(1)	2.691	C(5)-O(1)	2.719
O(3)-O(2)	2.505	C(3)-O(2)	2.391
H(32)-O(2)	2.578	H(33)-O(2)	2.578
H(41)-O(2)	1.998	H(42)-O(2)	1.998
C(5)-O(2)	2.689	C(5)-O(3)	2.737
C(6)-O(4)	2.913	H(61)-O(4)	2.689
C(7)-O(4)	2.361	C(10)-O(4)	2.712
H(101)-O(4)	2.370	C(10)-O(5)	2.980
H(112)-O(5)	2.687	H(152)-O(5)	2.634
C(16)-O(5)	2.846	H(161)-O(5)	2.478
H(21)-C(1)	1.985	H(22)-C(1)	1.984
H(12)-H(11)	1.568	H(13)-H(11)	1.568
C(2)-H(11)	1.985	H(21)-H(11)	2.274
H(22)-H(11)	2.274	H(13)-H(12)	1.568
C(2)-H(12)	1.985	H(22)-H(12)	2.274
C(2)-H(13)	1.985	H(21)-H(13)	2.274
H(22)-H(21)	1.568	H(41)-C(3)	1.989
H(42)-C(3)	1.989	H(32)-H(31)	1.568

H(33)-H(31)	1.568	C(4)-H(31)	1.991
H(41)-H(31)	2.278	H(42)-H(31)	2.278
H(33)-H(32)	1.568	C(4)-H(32)	1.991
H(42)-H(32)	2.279	C(4)-H(33)	1.991
H(41)-H(33)	2.279	H(42)-H(41)	1.568
H(61)-C(5)	2.010	H(62)-C(5)	2.010
C(7)-C(5)	2.569	H(71)-C(5)	2.680
H(71)-C(6)	2.036	C(8)-C(6)	2.478
H(62)-H(61)	1.568	C(7)-H(61)	2.045
C(8)-H(61)	2.679	C(7)-H(62)	2.044
C(8)-H(62)	2.570	C(9)-C(7)	2.938
C(8)-H(71)	2.102	C(9)-H(71)	2.533
C(9)-C(8)	2.472	C(10)-C(8)	2.433
H(101)-C(8)	2.492	C(10)-C(9)	2.492
C(15)-C(9)	2.837	H(152)-C(9)	2.568
C(16)-C(9)	2.455	H(161)-C(9)	2.513
H(111)-C(10)	2.053	H(112)-C(10)	2.053
C(12)-C(10)	2.506	H(121)-C(10)	2.709
C(13)-C(10)	2.920	C(14)-C(10)	2.492
H(142)-C(10)	2.696	H(151)-C(10)	2.050
H(152)-C(10)	2.050	C(11)-H(101)	2.033
H(111)-H(101)	2.321	C(12)-H(101)	2.660
C(14)-H(101)	2.651	C(15)-H(101)	2.031
H(151)-H(101)	2.317	H(121)-C(11)	2.050
H(122)-C(11)	2.050	C(13)-C(11)	2.495
H(132)-C(11)	2.701	C(14)-C(11)	2.920
C(15)-C(11)	2.509	H(152)-C(11)	2.713
H(112)-H(111)	1.568	C(12)-H(111)	2.049
H(121)-H(111)	2.321	H(122)-H(111)	2.353

C(12)-H(112)	2.049	H(122)-H(112)	2.321
C(13)-H(112)	2.699	C(15)-H(112)	2.705
H(131)-C(12)	2.042	H(132)-C(12)	2.041
C(14)-C(12)	2.504	H(142)-C(12)	2.713
C(15)-C(12)	2.949	H(122)-H(121)	1.568
C(13)-H(121)	2.044	H(131)-H(121)	2.309
C(14)-H(121)	2.717	C(13)-H(122)	2.045
H(131)-H(122)	2.349	H(132)-H(122)	2.307
H(141)-C(13)	2.040	H(142)-C(13)	2.040
C(15)-C(13)	2.517	H(152)-C(13)	2.731
H(132)-H(131)	1.568	C(14)-H(131)	2.040
H(141)-H(131)	2.341	H(142)-H(131)	2.304
C(14)-H(132)	2.040	H(141)-H(132)	2.304
C(15)-H(132)	2.724	H(151)-C(14)	2.059
H(152)-C(14)	2.058	H(142)-H(141)	1.568
C(15)-H(141)	2.053	H(152)-H(141)	2.325
C(15)-H(142)	2.053	H(151)-H(142)	2.322
H(152)-H(151)	1.568	H(171)-C(16)	2.043
H(172)-C(16)	2.043	C(18)-C(16)	2.516
H(182)-C(16)	2.735	C(19)-C(16)	2.940
C(20)-C(16)	2.501	H(201)-C(16)	2.710
H(211)-C(16)	2.037	H(212)-C(16)	2.037
C(17)-H(161)	2.036	H(171)-H(161)	2.305
C(18)-H(161)	2.708	C(20)-H(161)	2.678
C(21)-H(161)	2.018	H(212)-H(161)	2.287
H(181)-C(17)	2.052	H(182)-C(17)	2.052
C(19)-C(17)	2.513	H(192)-C(17)	2.733
C(20)-C(17)	2.921	C(21)-C(17)	2.496
H(211)-C(17)	2.708	H(172)-H(171)	1.568
C(18)-H(171)	2.053	H(182)-H(171)	2.309

C(18)-H(172)	2.053	H(181)-H(172)	2.309
C(19)-H(172)	2.736	C(21)-H(172)	2.715
H(191)-C(18)	2.035	H(192)-C(18)	2.035
C(20)-C(18)	2.489	H(201)-C(18)	2.702
C(21)-C(18)	2.925	H(182)-H(181)	1.568
C(19)-H(181)	2.032	H(191)-H(181)	2.343
H(192)-H(181)	2.291	C(19)-H(182)	2.032
H(191)-H(182)	2.289	C(20)-H(182)	2.706
H(201)-C(19)	2.042	H(202)-C(19)	2.042
C(21)-C(19)	2.515	H(211)-C(19)	2.726
H(192)-H(191)	1.568	C(20)-H(191)	2.044
H(201)-H(191)	2.304	H(202)-H(191)	2.349
C(20)-H(192)	2.044	H(202)-H(192)	2.305
C(21)-H(192)	2.730	H(211)-C(20)	2.051
H(212)-C(20)	2.051	H(202)-H(201)	1.568
C(21)-H(201)	2.049	H(212)-H(201)	2.315
C(21)-H(202)	2.049	H(211)-H(202)	2.316
H(212)-H(202)	2.352	H(212)-H(211)	1.568

Intermolecular:

H(42)-F(1a)	2.612	O(3)-N(5b)	2.971
O(3)-H(5b)	2.018	H(62)-O(3b)	2.352
H(142)-O(4c)	2.595	H(71)-O(5d)	2.697

Key to symmetry operations relating
designated atoms to reference atoms
at (x,y,z):

(a) 1.0-x,1.0-y,1.0-z

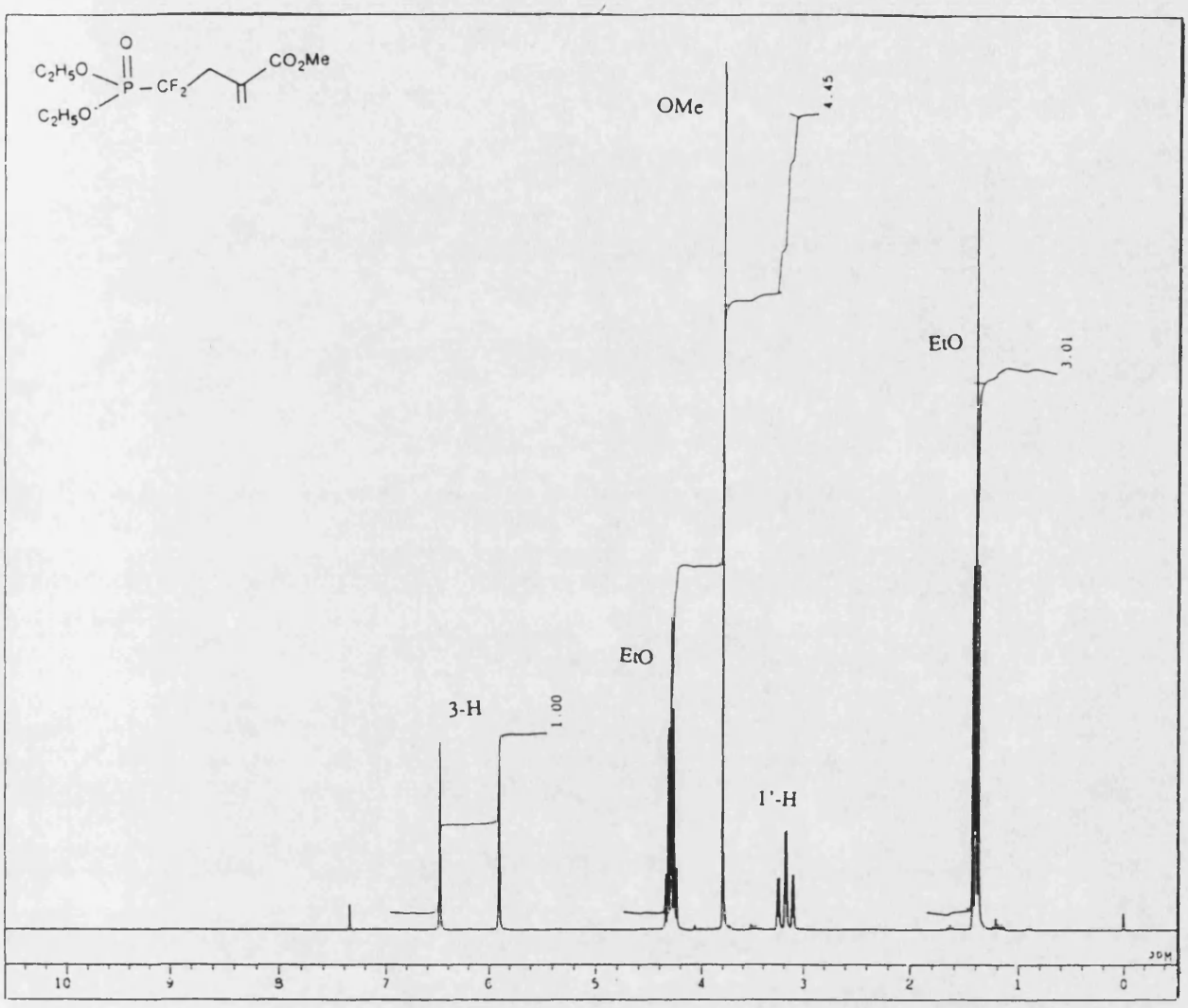
(b) 1.0-x,2.0-y,1.0-z

(c) 2.0-x,2.0-y,1.0-z

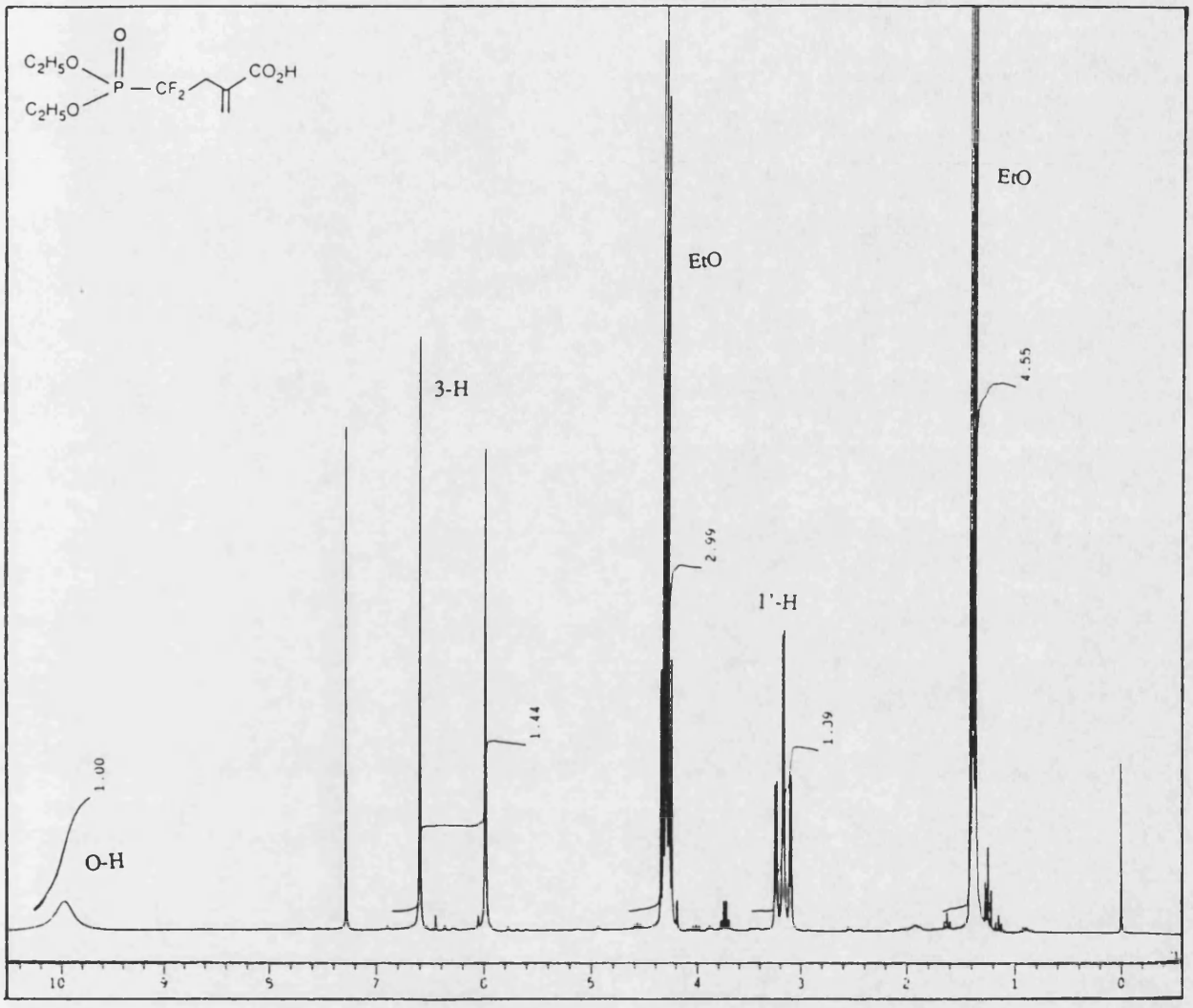
(d) 2.0-x,2.0-y,2.0-z

APPENDIX THREE

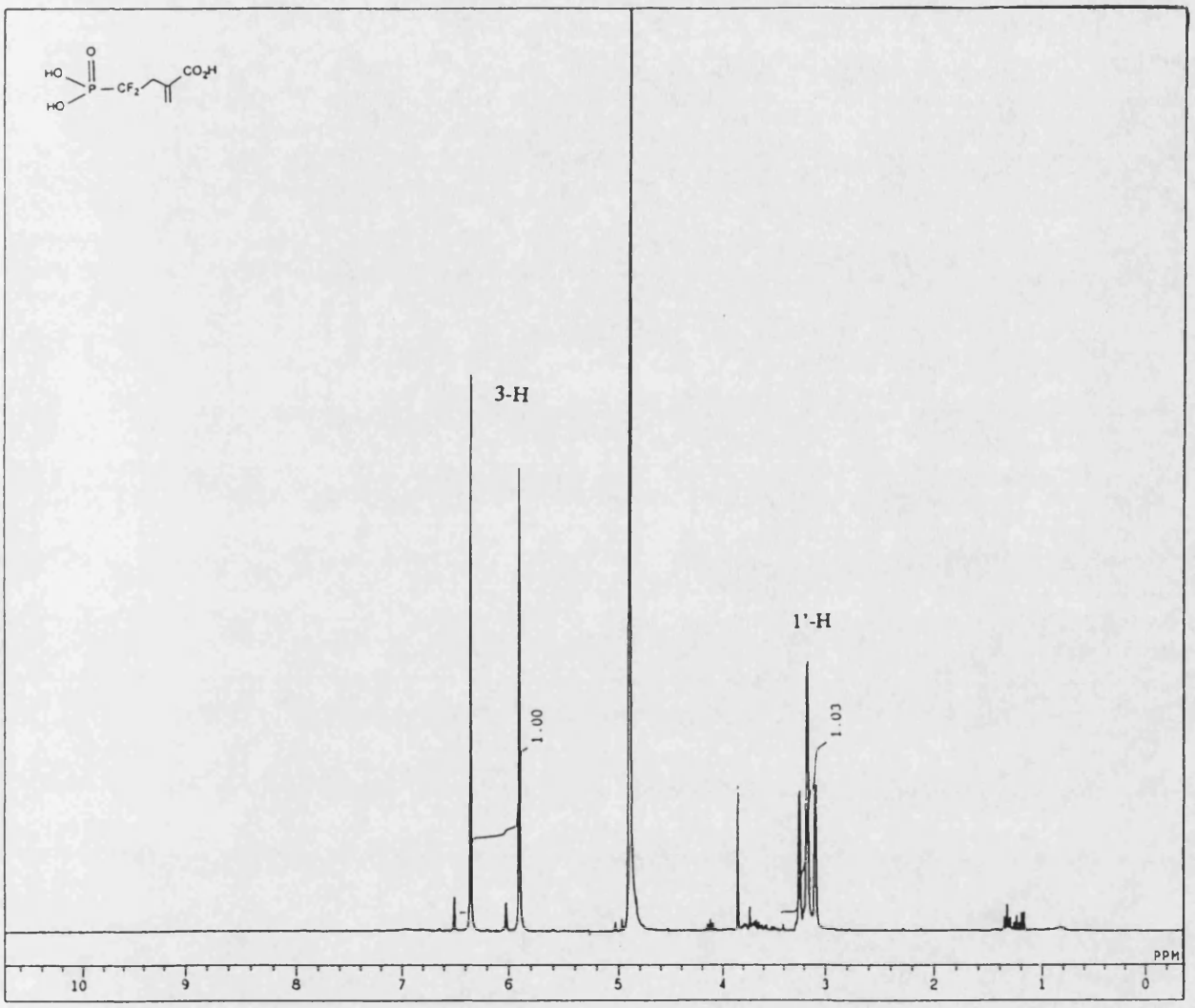
SELECTED NMR SPECTRA



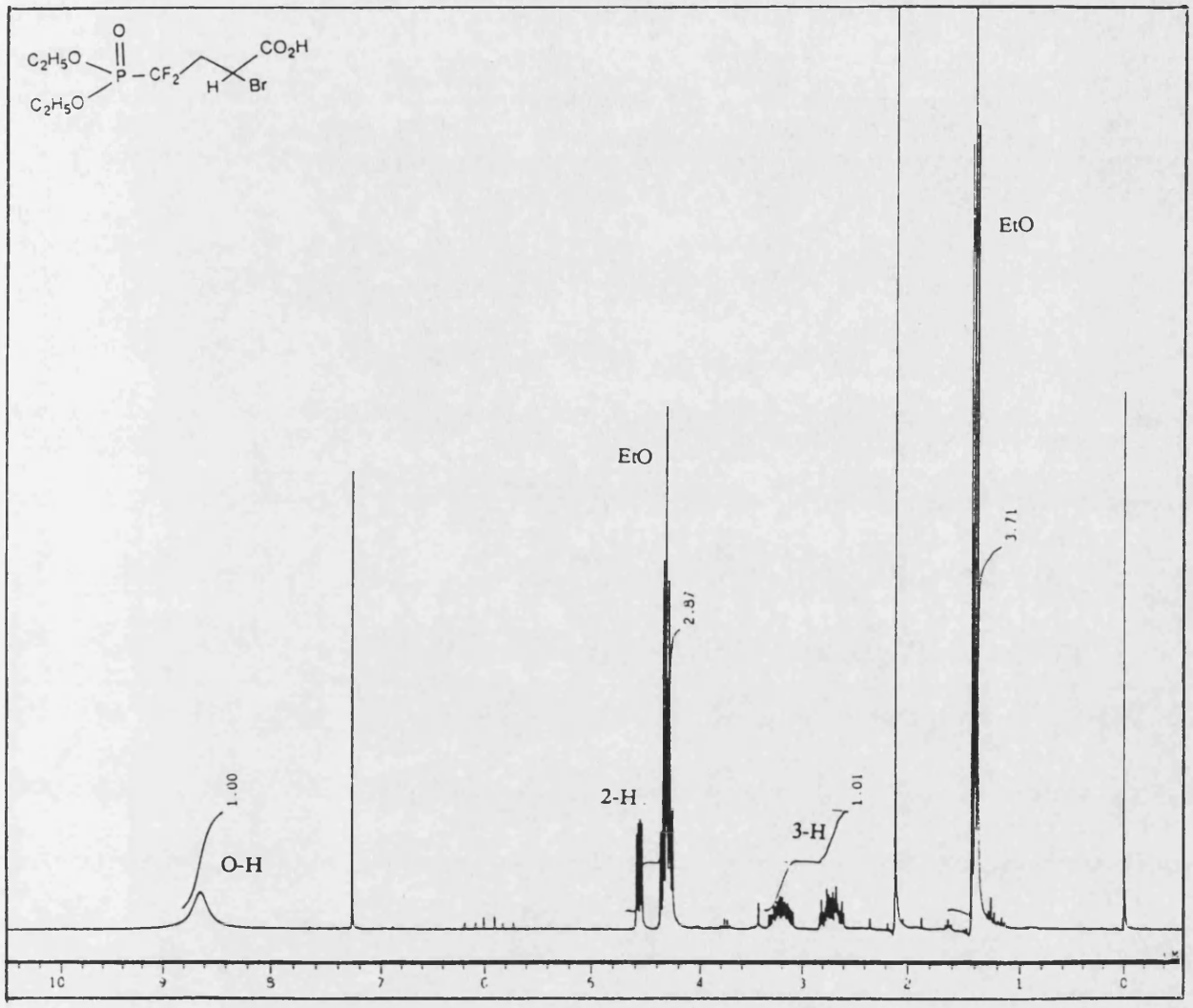
^{1}H NMR Spectrum of (67) (270 MHz, $CDCl_3$)



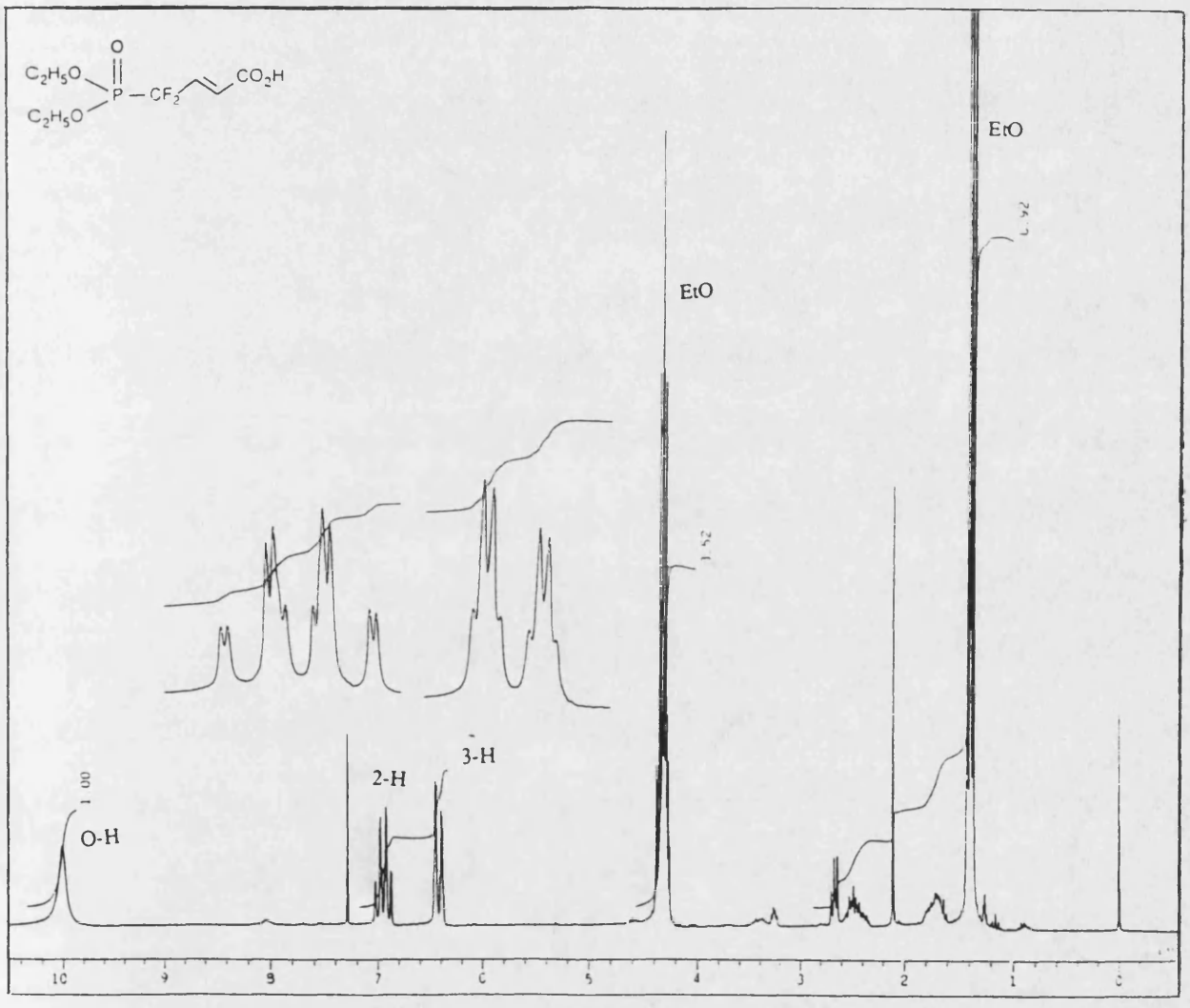
^{1}H NMR Spectrum of (68) (270 MHz, $CDCl_3$)



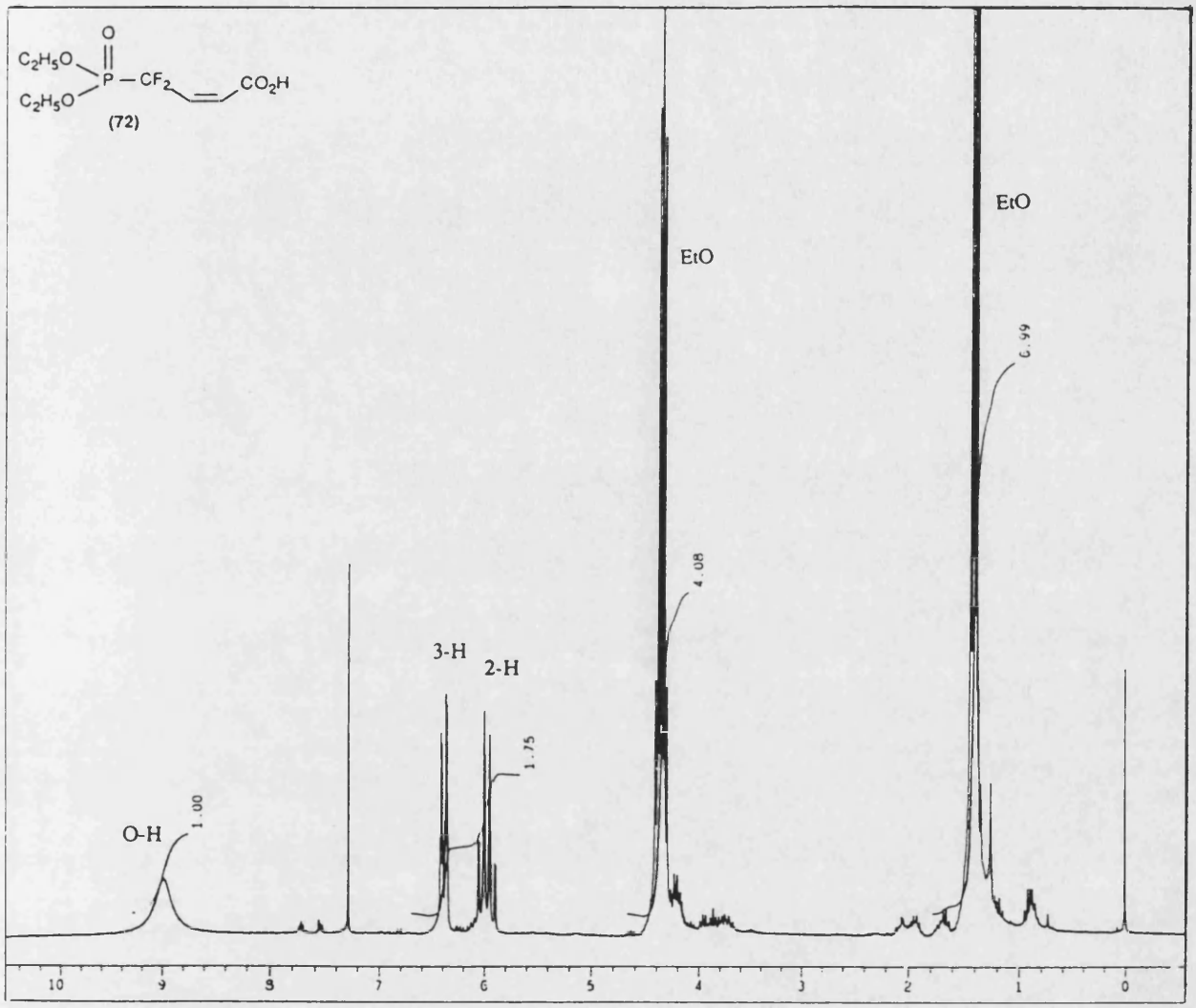
^{1}H NMR Spectrum of (69) (270 MHz, $CDCl_3$)



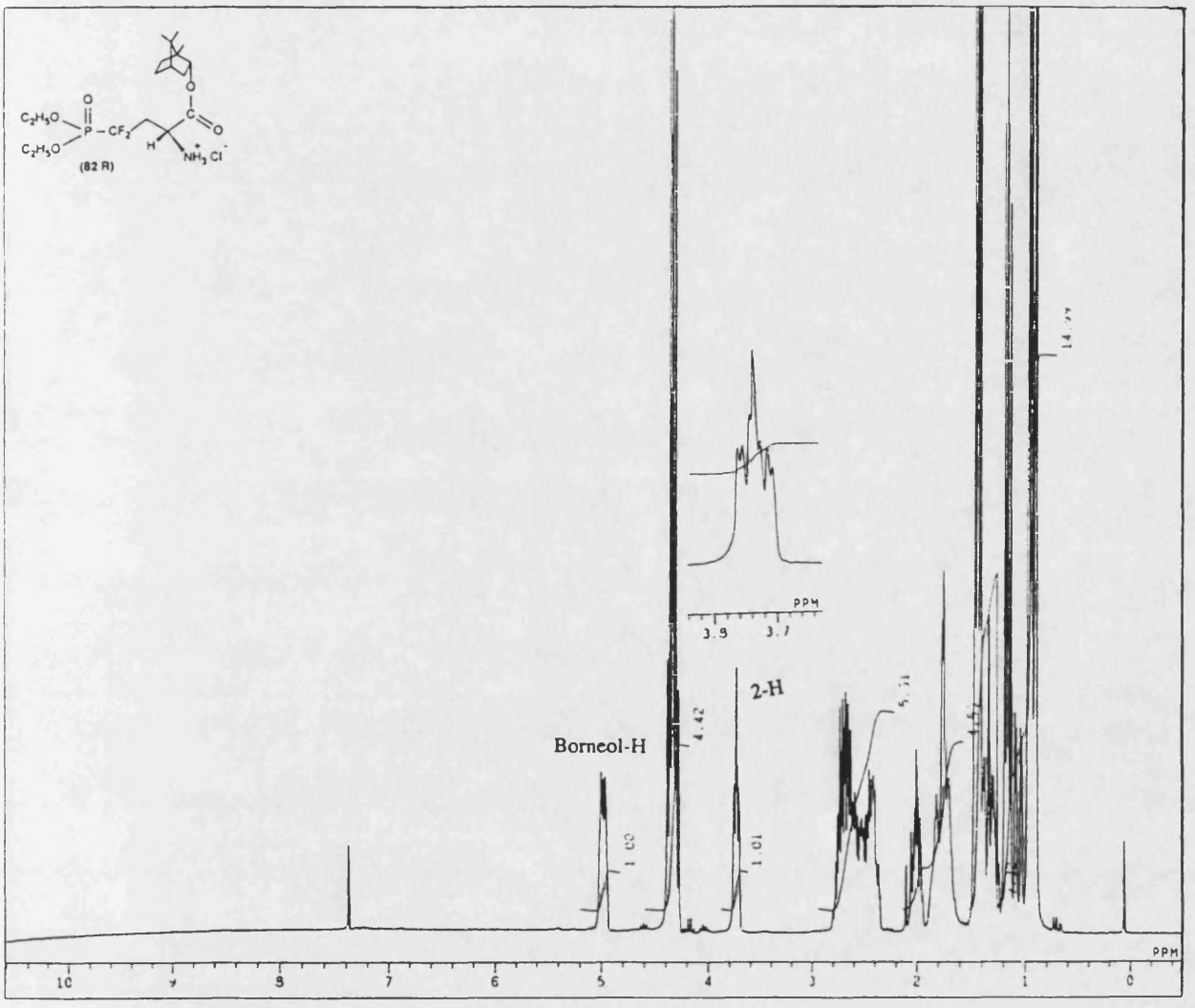
^{1}H NMR Spectrum of (70) (270 MHz, $CDCl_3$)



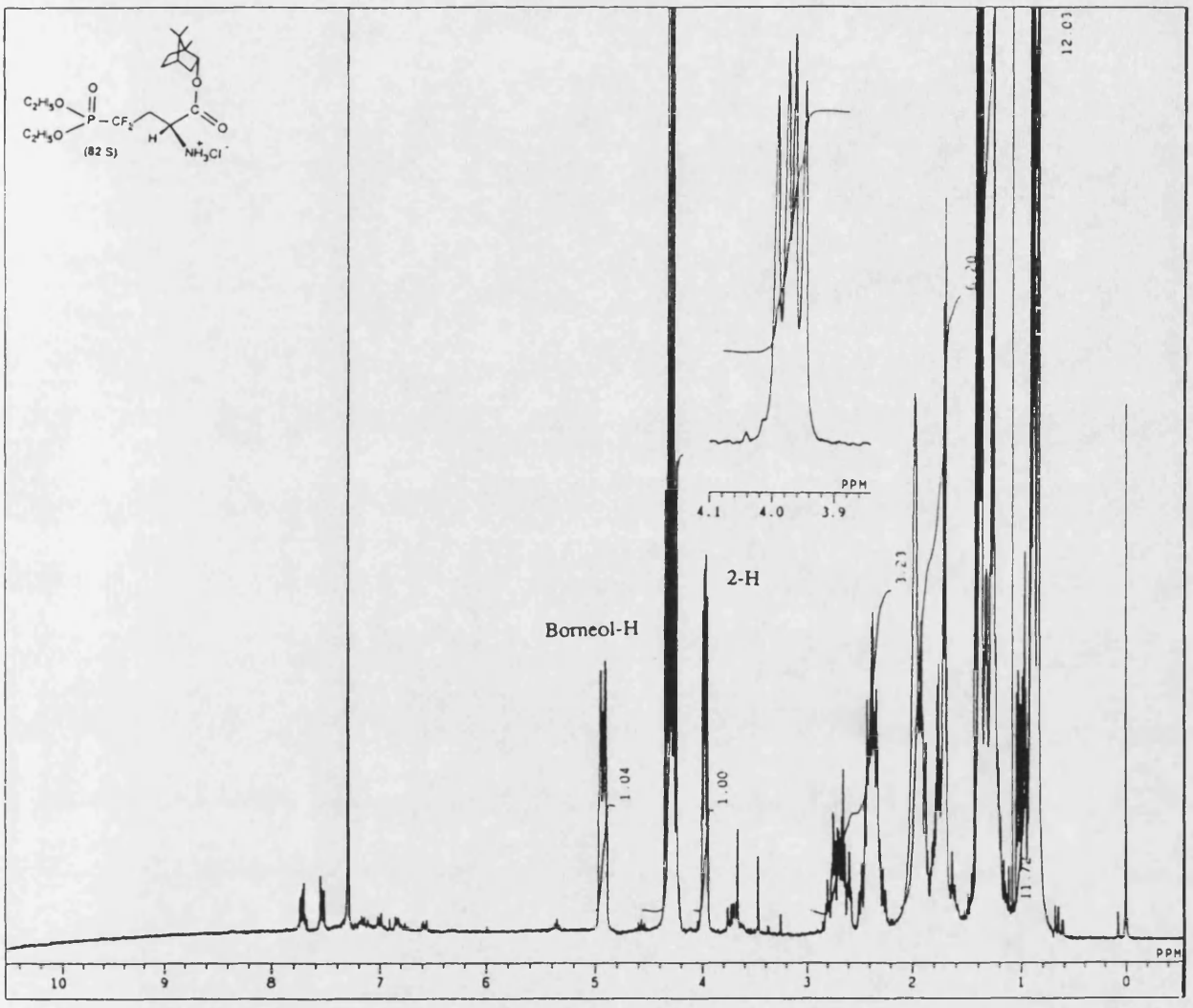
^{1}H NMR Spectrum of (71) (270 MHz, $CDCl_3$)



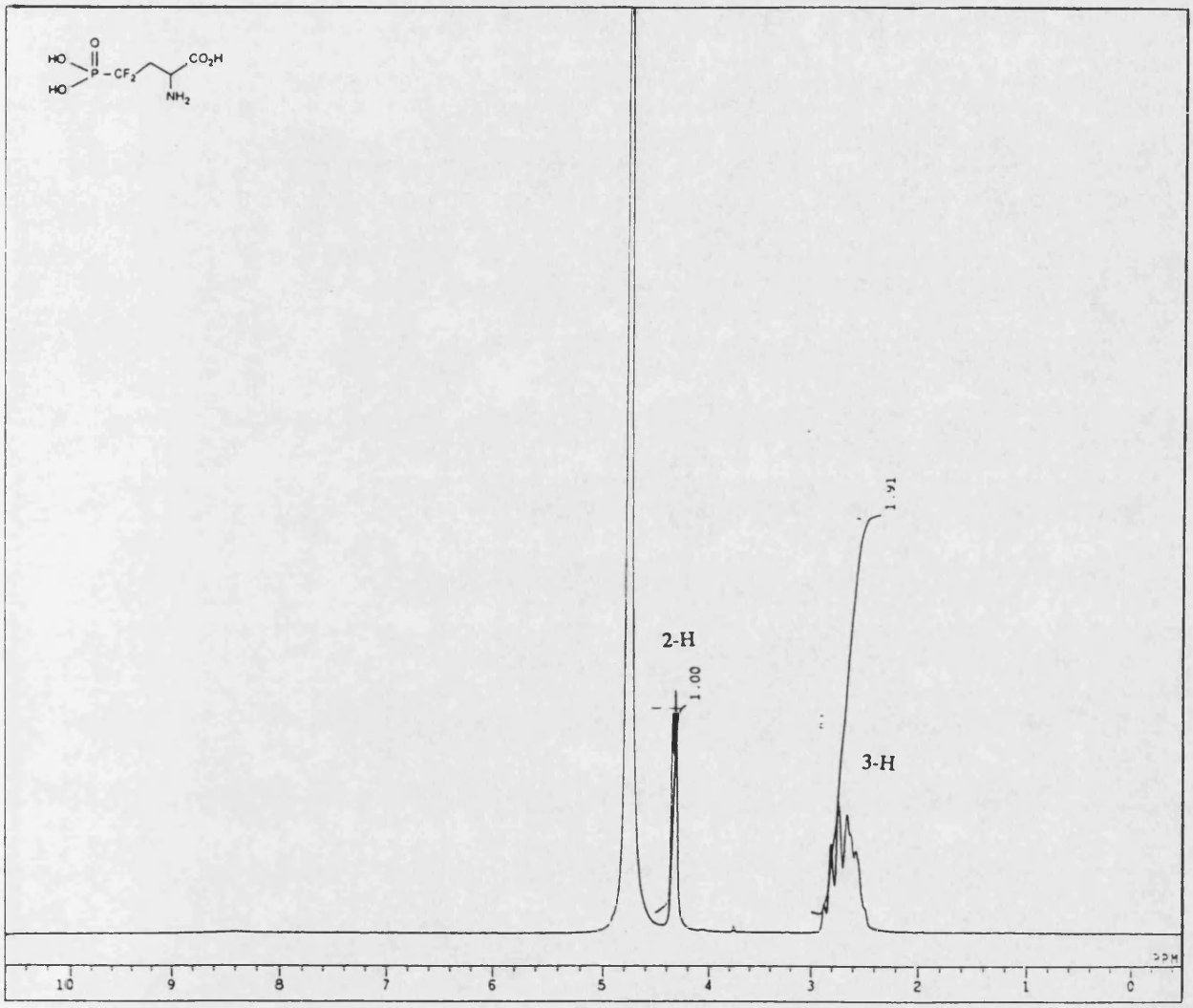
^{1}H NMR Spectrum of (72) (270 MHz, $CDCl_3$)



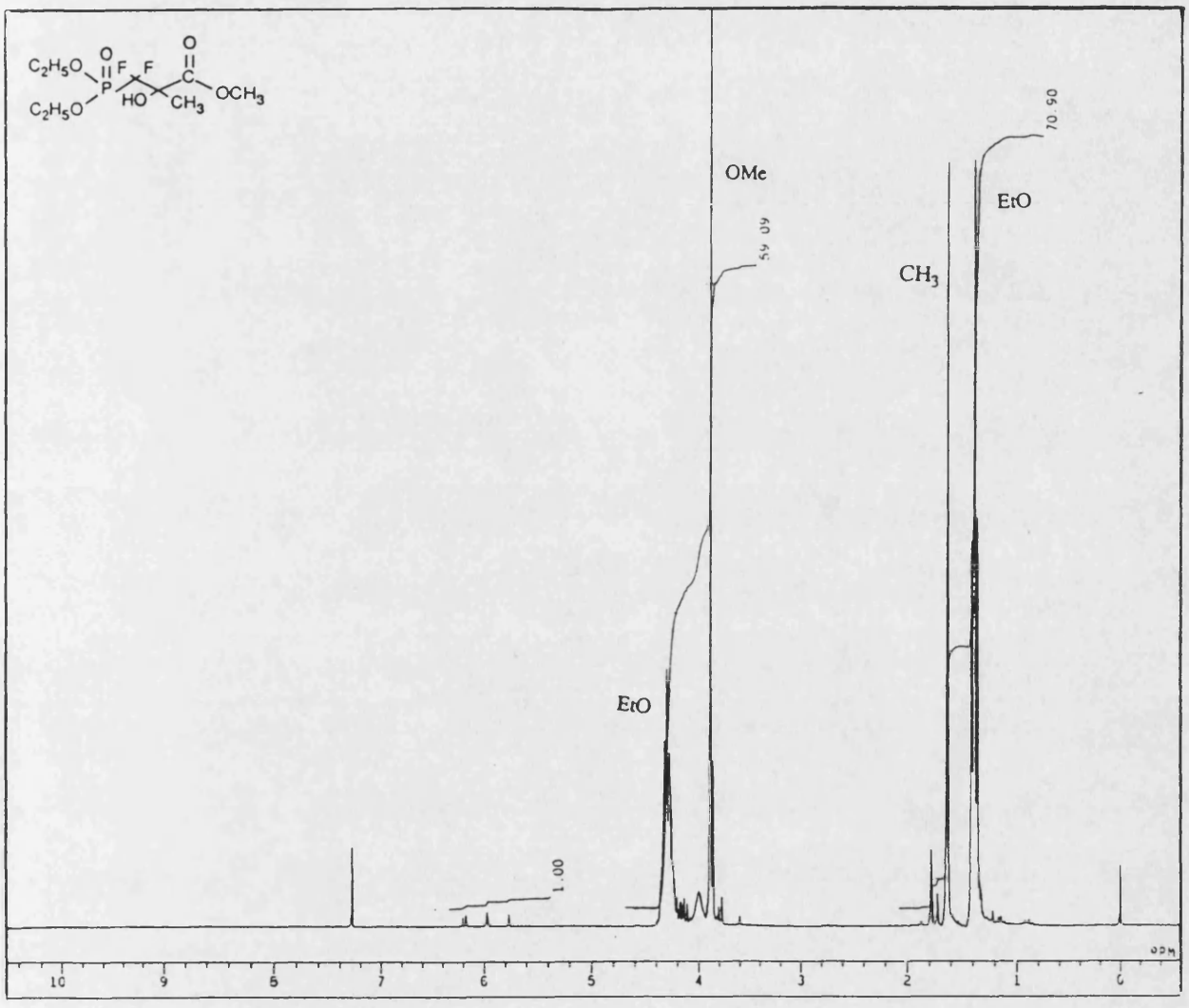
^{1}H NMR Spectrum of (82 R) (270 MHz, $CDCl_3$)



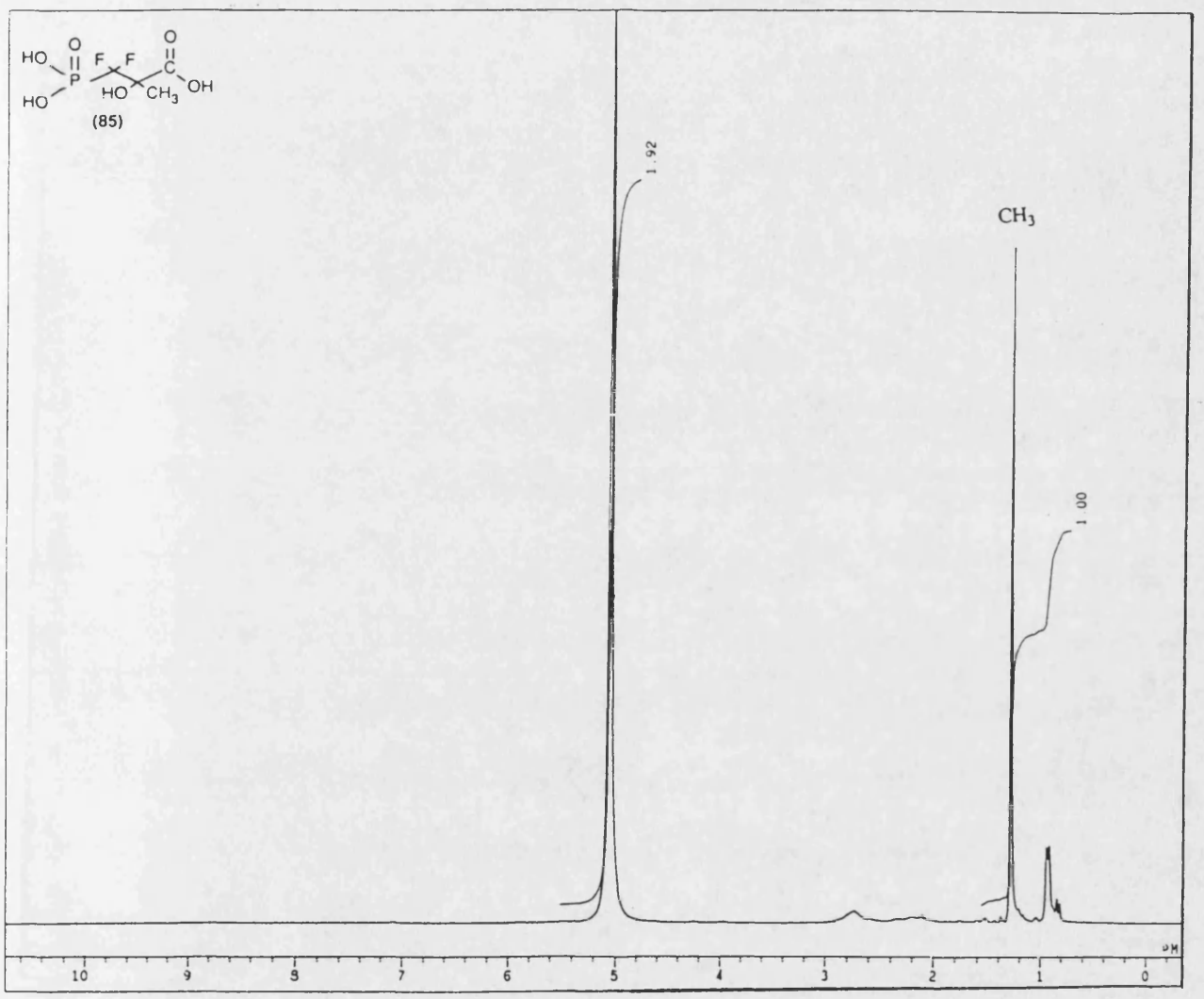
^{1}H NMR Spectrum of (82 S) (270 MHz, $CDCl_3$)



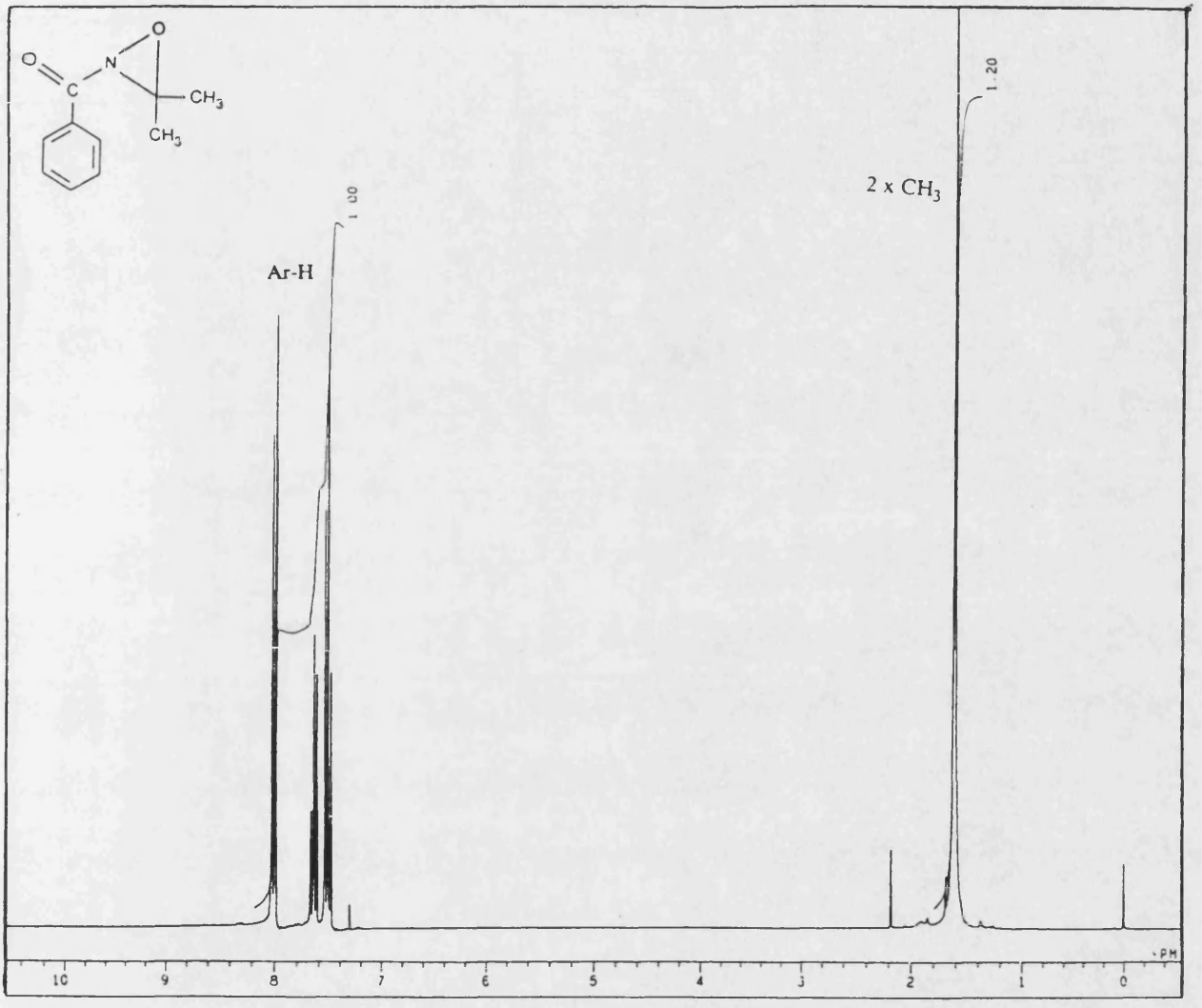
^{1}H NMR Spectrum of (76) (270 MHz, D_2O)



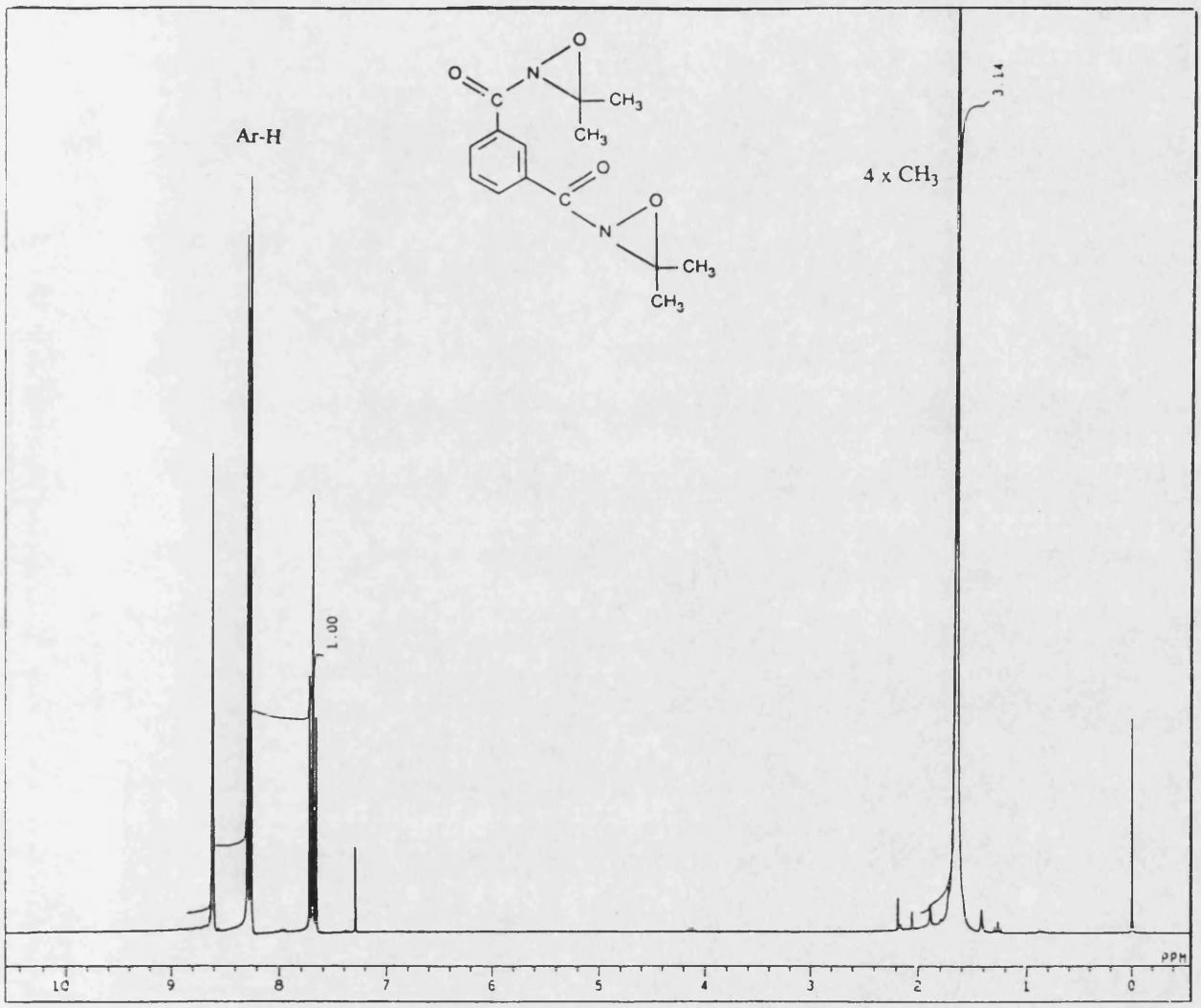
^{1}H NMR Spectrum of (84) (270 MHz, $CDCl_3$)



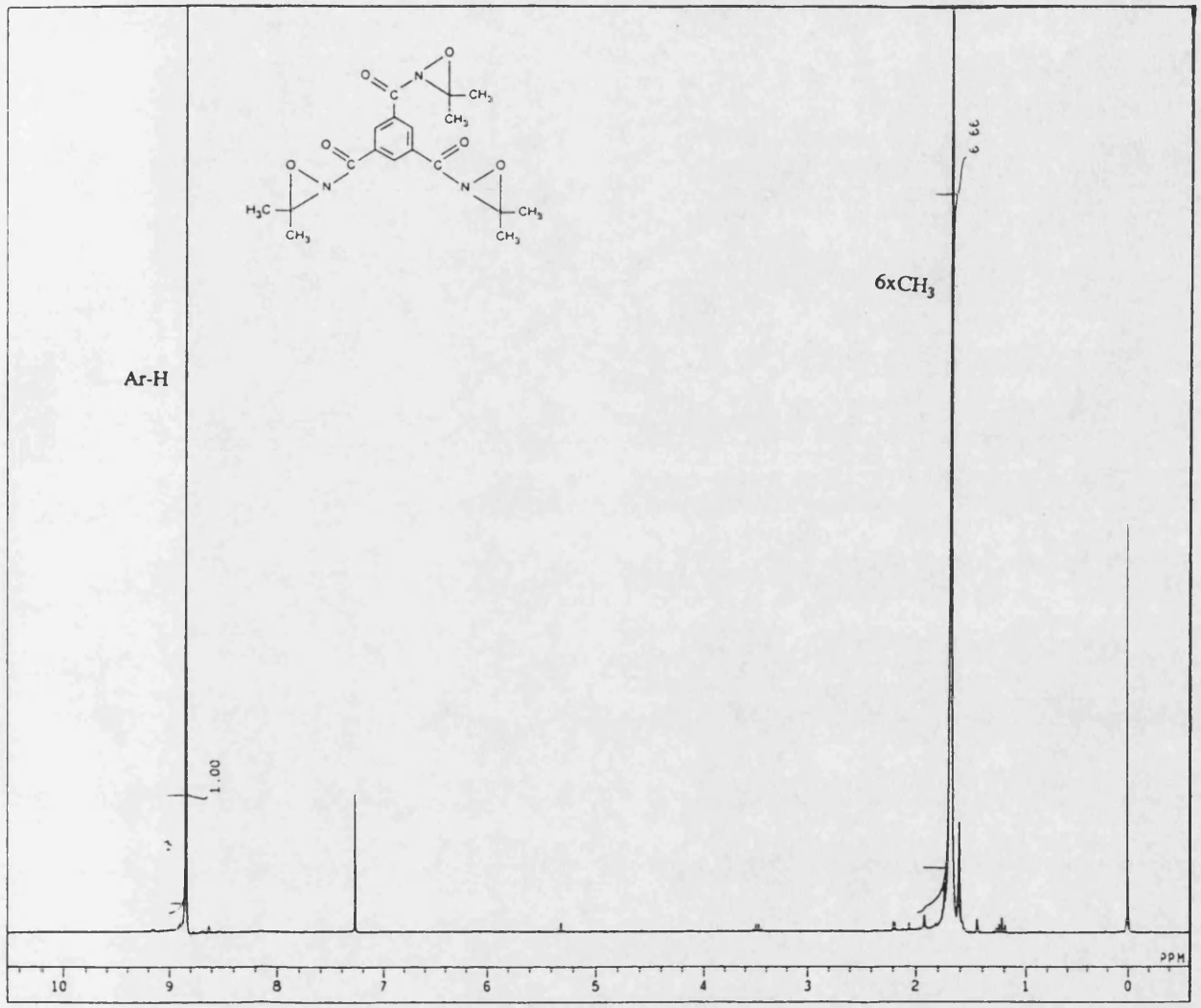
^{1}H NMR Spectrum of (85) (270 MHz, $CDCl_3$)



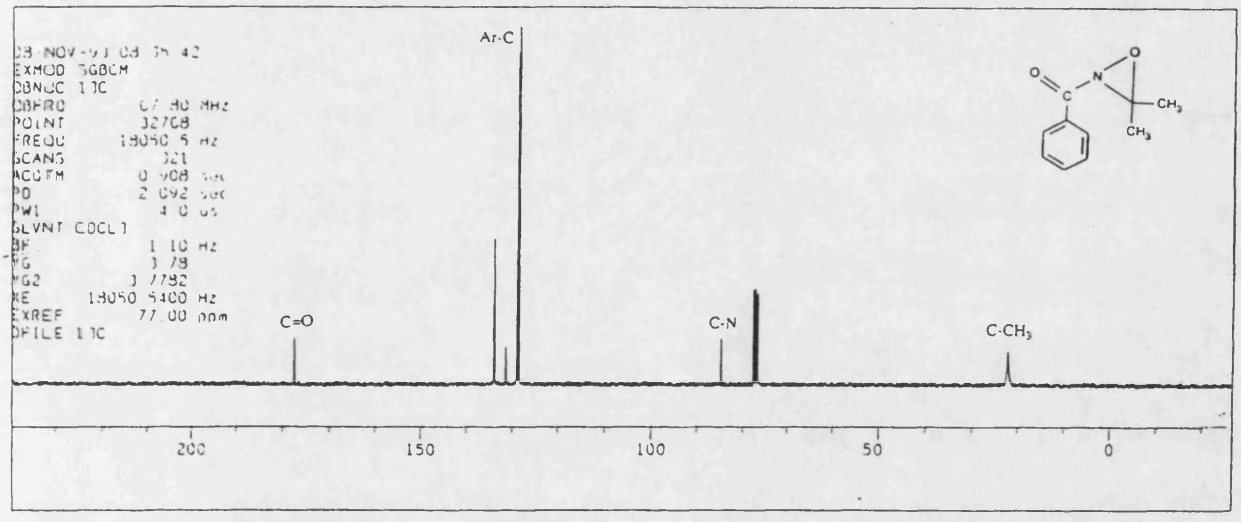
^{1}H NMR Spectrum of (113) (270 MHz, $CDCl_3$)



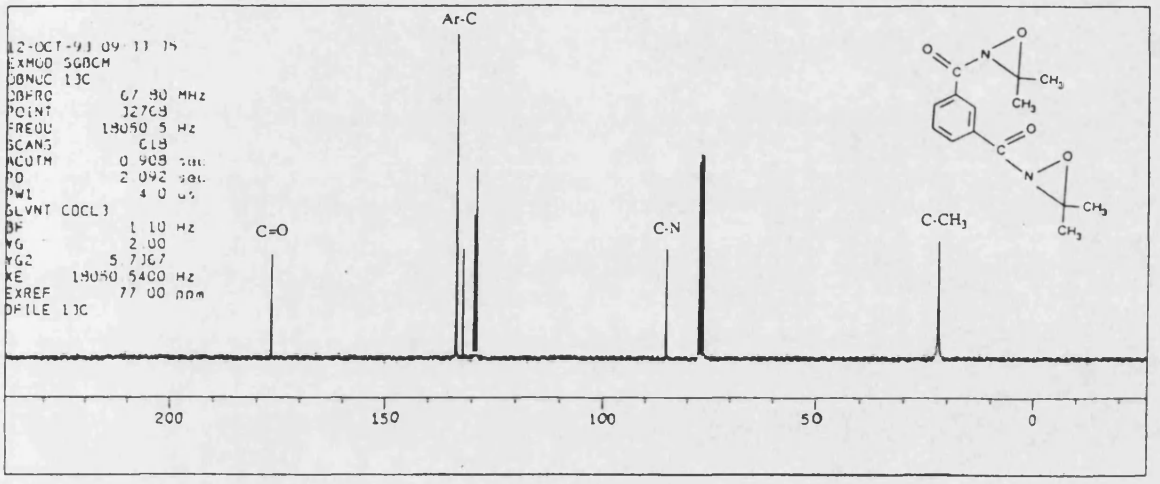
^{1}H NMR Spectrum of (114) (270 MHz, $CDCl_3$)



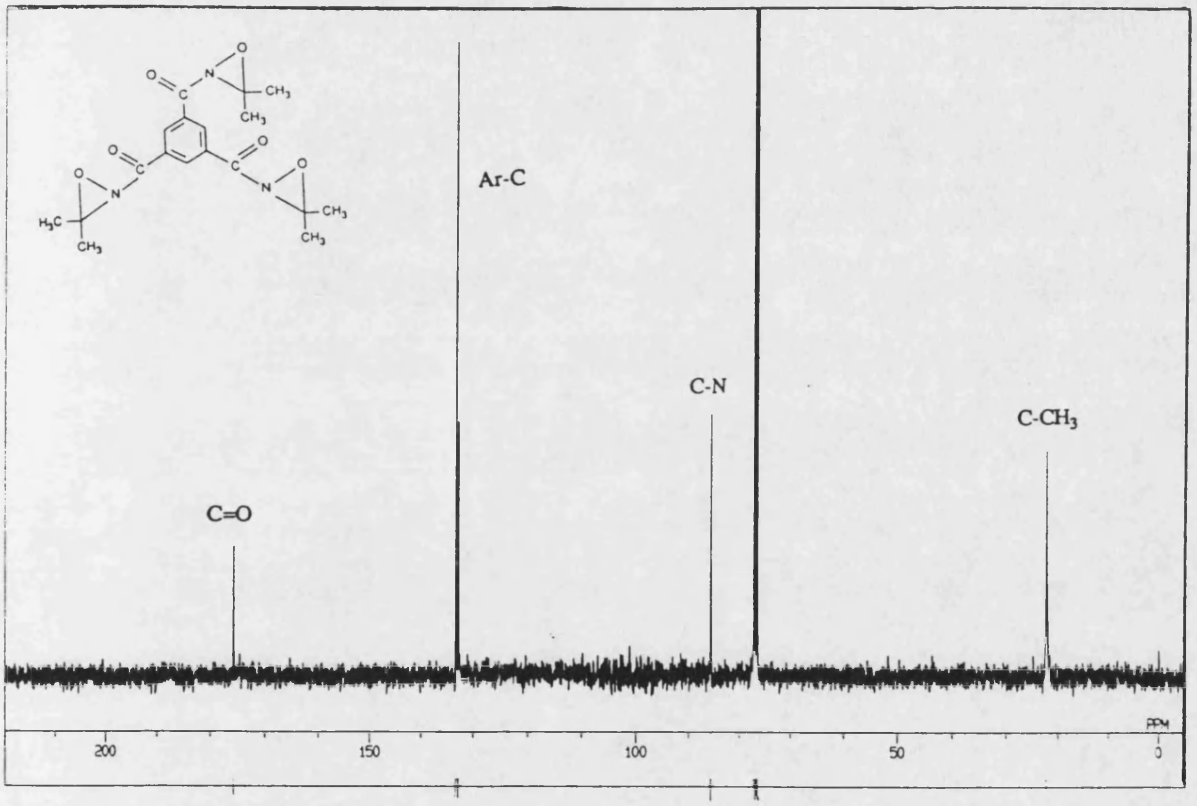
^{1}H NMR Spectrum of (115) (270 MHz, $CDCl_3$)



^{13}C NMR Spectrum of (113) (270 MHz, $CDCl_3$)



^{13}C NMR Spectrum of (114) (270 MHz, $CDCl_3$)



^{13}C NMR Spectrum of (115) (270 MHz, $CDCl_3$)